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(71) Applicant *(for all designated States except US)*: **IMMUNEX CORPORATION** [US/US]; 51 University Street, Seattle, WA 98101 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **FANSLOW, William, C., III** [US/US]; 404 S.W. 197th Street, Normandy Park, WA 98166 (US). **CERRETTI, Douglas, Pat** [US/US]; 1607 North 197th Place, Seattle, WA 98133 (US). **POINDEXTER, Kurt, Matthew** [US/US]; 9247 Interlake Avenue North, Apt. 2, Seattle, WA 98103 (US). **BLACK, Roy, A.** [US/US]; 8062 30th Avenue Northeast, Seattle, WA 98115 (US).

(74) Agent: **SMITH, Julie, K.**; 51 University Street, Seattle, WA 98101 (US).

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(54) Title: INTEGRIN ANTAGONISTS

(57) Abstract: The present invention provides methods and compositions for inhibiting the biological activity of integrins, for inhibiting endothelial cell migration, and for inhibiting angiogenesis. In particular, the invention provides compositions comprising ADAM disintegrin domains and methods for using said compositions. In preferred embodiments the methods and compositions of the invention are used to inhibit angiogenesis and to treat diseases or conditions mediated by angiogenesis.

# TITLE

INTEGRIN ANTAGONISTS

## CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of pending U.S. provisional application Serial No. 60/184,865, filed 25 February 2000, the contents of which are incorporated herein by reference.

## FIELD OF THE INVENTION

This invention relates to methods and compositions that are useful for antagonizing the interaction between integrins and their ligands. In particular, the invention relates to the use of ADAM disintegrin domains for antagonizing the interaction between integrins and their ligands.

## BACKGROUND OF THE INVENTION

### A. Integrins and Disintegrins

Integrins are a family of cell surface proteins that mediate adhesion between cells (cell-cell adhesion) and between cells and extracellular matrix proteins (cell-ECM adhesion). Integrins are heterodimeric structures composed of noncovalently bound α and β subunits. In humans, at least fifteen different α subunits and eight different β subunits combine to form integrins with diverse biological activities and ligand specificities. Integrins play important roles in biological processes including embryonic development, platelet aggregation, immune reactions, tissue repair and remodeling, bone resorption, and tumor invasion and metastasis. Integrins are, therefore, important targets for therapeutic intervention in human disease.

The disintegrins are a family of low molecular weight, soluble, cysteine-rich peptides which have been isolated from snake venom (reviewed in Niewiarowski et al., Seminars in Hematology 31(4):289, 1994). The snake venom disintegrins typically contain an RGD (Arg-Gly-Asp, SEQ ID NO:19) motif. The RGD motif is recognized by many integrins, and is present in several integrin ligands including fibronectin, vitronectin, and von Willebrand factor. Disintegrins disrupt normal adhesion processes by inhibiting the binding of cell surface integrins to their ligands.

Disintegrin-like domains have been identified in cellular proteins from both invertebrates and vertebrates (see, e.g., Westcamp and Blobel, Proc. Natl. Acad. Sci. USA 91:2748, 1994; Wolfsberg et al., Dev. Biol. 169:378, 1995; Alfandari et al., Dev. Biol. 182:314, 1997), including the ADAM family of transmembrane proteins.

### B. ADAMs

The ADAMs, which have also been called MDCs, are a family of type I transmembrane cysteine-rich glycoproteins (Weskamp et al., Proc. Natl. Acad. Sci. USA, 91:2748, 1994; Wolfsberg et al., Dev. Biol. 169:378, 1995). The multidomain structure of the ADAMs typically includes an amino-terminal metalloprotease domain, a disintegrin domain, a cysteine-rich region (the region between the

disintegrin domain and the transmembrane domain), a transmembrane region, and a cytoplasmic domain. At least 30 ADAM family members have been identified, in a variety of animal species. The structure of the ADAMs suggests that they may be involved in a variety of biological processes, including cell adhesion, cell fusion, signal transduction, and proteolysis. Members of the ADAM family have, in fact, been shown to play roles in sperm-egg binding and fusion, myotube formation, neurogenesis, and proteolysis.

ADAM-15, also called MDC-15 or metargidin, is the only ADAM identified to date which contains an RGD motif within its disintegrin domain. Zhang et al. (J. Biol. Chem. 273(13):7345, 1998) have reported that the isolated disintegrin domain of ADAM-15, expressed in E. coli as a glutathione S-transferase fusion protein, specifically interacts with $\alpha_v\beta_3$ integrin and that the interaction is mediated by the RGD tripeptide sequence. The recombinant fusion protein did not interact with other integrins tested, including $\alpha_{IIb}\beta_3$ and $\alpha_5\beta_1$. Nath et al. (J. Cell Science 112:579, 1999) have reported that the entire ADAM-15 extracellular domain, expressed as an Fc fusion protein in COS cells, interacts with $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrins on hematopoietic cells and that the interaction is mediated by the RGD tripeptide sequence. Zhang et al. and Nath et al. commented that the RGD-dependent interaction between ADAM-15 and $\alpha_v\beta_3$ integrin suggests a role in processes such as malignancy and angiogenesis.

C. Angiogenesis

Angiogenesis, the generation of new blood vessels, is a spatially and temporally regulated process in which endothelial and smooth muscle cells proliferate, migrate, and assemble into tubes, in response to endogenous positive and negative regulatory molecules. Angiogenesis plays important roles in both normal and pathological physiology.

Under normal physiological conditions, angiogenesis is involved in fetal and embryonic development, wound healing, organ regeneration, and female reproductive remodeling processes including formation of the endometrium, corpus luteum, and placenta. Angiogenesis is stringently regulated under normal conditions, especially in adult animals, and perturbation of the regulatory controls can lead to pathological angiogenesis.

Pathological angiogenesis has been implicated in the manifestation and/or progression of inflammatory diseases, certain eye disorders, and cancer. In particular, several lines of evidence support the concept that angiogenesis is essential for the growth and persistence of solid tumors and their metastases (see, e.g., Folkman, N. Engl. J. Med. 285:1182, 1971; Folkman et al., Nature 339:58, 1989; Kim et al., Nature 362:841, 1993; Hori et al., Cancer Res., 51:6180, 1991; Zetter, Annu. Rev. Med. 49:407, 1998). The formation of new blood vessels provides a growing tumor with oxygen, nutrients, waste removal, and a conduit by which invasive cells can enter the circulatory system and establish distant metastases. Various classes of angiogenesis inhibitors are presently being developed and tested for the prevention (e.g., treatment of premalignant conditions), intervention (e.g., treatment of small tumors), and regression (e.g., treatment of large tumors) of cancers (see, e.g., Bergers et al.,

Science 284:808, 1999) and other forms of pathological angiogenesis. Because many steps in the angiogenic process, including endothelial cell migration, proliferation, and morphogenesis require vascular cell adhesion, certain integrin antagonists have been tested as anti-angiogenic agents.

Several integrins are expressed on the surface of cultured endothelial and smooth muscle cells, including $\alpha_v\beta_3$ integrin. The $\alpha_v\beta_3$ integrin is an endothelial cell receptor for von Willebrand factor, fibrin, fibrinogen, and fibronectin, and a marker of angiogenic vascular tissue. Brooks et al. have reported that monoclonal antibodies to $\alpha_v\beta_3$ integrin, as well as cyclic peptide inhibitors, disrupt angiogenesis and that $\alpha_v\beta_3$ antibodies promote tumor regression (Science 264:569, 1994; Cell 79:1157, 1994). These results suggest that $\alpha_v\beta_3$ integrin is a useful therapeutic target for diseases characterized by pathological angiogenesis.

There is great need for additional compositions and methods of antagonizing the interaction between integrins and their ligands. In particular, there is great need for additional compositions and methods of inhibiting angiogenesis for the prevention, abrogation, and mitigation of disease processes that are dependent upon pathological angiogenesis.

## SUMMARY OF THE INVENTION

The present invention is based upon the discovery that ADAM disintegrin domains are useful for inhibiting the biological activity of integrins and for inhibiting endothelial cell migration and angiogenesis, including the unexpected discovery that these inhibitory activities reside in ADAM disintegrin domains that lack an RGD motif.

The invention is directed to methods of antagonizing the binding of an integrin to its ligands, and thereby inhibiting the biological activity of the integrin, comprising contacting the integrin with an effective amount of an ADAM disintegrin domain polypeptide. The invention is further directed to methods of inhibiting endothelial cell migration and methods of inhibiting angiogenesis comprising administering an effective amount of an ADAM disintegrin domain polypeptide. In some embodiments the ADAM disintegrin domain polypeptide is in the form of a multimer, preferably a leucine zipper multimer or Fc polypeptide. In some embodiments the ADAM disintegrin domain is from a human ADAM, and preferably from ADAM-8, ADAM-9, ADAM-10, ADAM-15, ADAM-17, ADAM-20, ADAM-21, ADAM-22, ADAM-23, or ADAM-29. The ADAM disintegrin domain is preferably produced in a recombinant cell, and is preferably present in a composition comprising a pharmaceutically acceptable carrier.

In some preferred embodiments the ADAM disintegrin domain polypeptide comprises an amino acid sequence selected from the group consisting of: amino acids 23-264 of SEQ ID NO:2, amino acids 23-303 of SEQ ID NO:4, amino acids 23-235 of SEQ ID NO:6, amino acids 23-292 of SEQ ID NO:8, amino acids 23-216 of SEQ ID NO:10, amino acids 23-305 of SEQ ID NO:12, amino acids 23-293 of SEQ ID NO:14, amino acids 23-312 of SEQ ID NO:16, amino acids 23-310 of SEQ ID NO:18, and amino acids 23-298 of SEQ ID NO:22. In some more preferred embodiments the ADAM disintegrin domain polypeptide comprises an amino acid sequence selected from the group

consisting of: amino acids 34-91 of SEQ ID NO:2, amino acids 34-92 of SEQ ID NO:4, amino acids 34-99 of SEQ ID NO:6, amino acids 34-92 of SEQ ID NO:8, amino acids 34-93 of SEQ ID NO:10, amino acids 34-91 of SEQ ID NO:12, amino acids 34-91 of SEQ ID NO:14, amino acids 34-92 of SEQ ID NO:16, amino acids 34-91 of SEQ ID NO:18, and amino acids 34-91 of SEQ ID NO:22. In some most preferred embodiments the ADAM disintegrin domain polypeptide comprises an amino acid sequence selected from the group consisting of: amino acids 78-91 of SEQ ID NO:2, amino acids 79-92 of SEQ ID NO:4, amino acids 87-99 of SEQ ID NO:6, amino acids 79-92 of SEQ ID NO:8, amino acids 79-93 of SEQ ID NO:10, amino acids 78-91 of SEQ ID NO:12, amino acids 78-91 of SEQ ID NO:14, amino acids 79-92 of SEQ ID NO:16, amino acids 78-91 of SEQ ID NO:18, and amino acids 78-91 of SEQ ID NO:22.

In some embodiments a therapeutically effective amount of the ADAM disintegrin domain is administered to a mammal in need of such treatment. In preferred embodiments the mammal is afflicted with a condition mediated by angiogenesis, an ocular disorder, malignant or metastatic condition, inflammatory disease, osteoporosis and other conditions mediated by accelerated bone resorption, restenosis, inappropriate platelet activation, recruitment, or aggregation, thrombosis, or a condition requiring tissue repair or wound healing. The ADAM disintegrin domain is, in some embodiments, administered in combination with radiation therapy and/or in combination with one or more additional therapeutic agents.

The invention also encompasses methods for identifying compounds that modulate integrin biological activity, that modulate the interaction between an integrin and an ADAM disintegrin domain, that inhibit endothelial cell migration, or that inhibit angiogenesis, comprising combining a test compound with an integrin or with endothelial cells and with an ADAM disintegrin domain polypeptide that binds to the integrin or endothelial cells and determining whether the test compound alters the binding of the ADAM disintegrin domain polypeptide to the integrin or endothelial cells.

These and other aspects of the present invention will become evident upon reference to the following detailed description, examples, and claims.

## DETAILED DESCRIPTION OF THE INVENTION

A. Abbreviations and Terminology Used in the Specification

"4-1BB" and "4-1BB ligand" (4-1BB-L) are polypeptides described, inter alia, in U.S. Patent No. 5,674,704, including soluble forms thereof.

"ADAMs" are a family of transmembrane glycoproteins having disintegrin and metalloproteinase domains, also called MDC, metalloprotease/disintegrin/cysteine-rich proteins.

"Dis" is a disintegrin domain; "ADAMdis" is an ADAM disintegrin domain.

"CD40 ligand" (CD40L) is a polypeptide described, inter alia, in U.S. Patent No. 5,716.805, including soluble forms thereof.

"CD148" is a protein tyrosine phosphatase, also called DEP-1, ECRTP, and PTPRJ. CD148 binding proteins are described in Daniel et al., PCT Publication No. WO 00/15258, 23 March 2000.

"DMEM" is Dulbecco's Modified Eagle Medium.

"FACS" is fluorescence activated cell sorting.

"Flt3L" is Flt3 ligand, a polypeptide described, inter alia, in U.S. Patent No. 5,554,512, including soluble forms thereof.

"HRMEC" are human renal microvascular endothelial cells.

"HMVEC-d" are human dermal microvascular endothelial cells.

"mAb" is a monoclonal antibody.

"MDC" is a family of cysteine-rich proteins having metalloprotease and disintegrin domains, also called ADAM.

"Nectin-3" is a cell adhesion molecule in the nectin family (which is described, inter alia, in Satoh-Horikawa et al., J. Biol. Chem. 275(14):10291, 2000). The GenBank accession numbers of human nectin-3 nucleic acid and polypeptide sequences are AF282874 and AAF97597 respectively (Reymond et al., 2000).

"PMA" is phorbol-12-myristate-13-acetate.

"Tek," which has also been called Tie2 and ork, is an receptor tyrosine kinase (RTK) that is predominantly expressed in vascular endothelium. The molecular cloning of human Tek (ork) has been described by Ziegler, U.S. Patent No. 5,447,860. "Tek antagonists" are described, inter alia, in Cerretti et al., PCT Publication No. WO 00/75323, 14 December 2000.

"TNF" is tumor necrosis factor. "TNFR" is a tumor necrosis factor receptor, including soluble forms thereof. "TNFR/Fc" is a tumor necrosis factor receptor-Fc fusion polypeptide.

"TRAIL" is TNF-related apoptosis-inducing ligand, a type II transmembrane polypeptide in the TNF family described, inter alia, in U.S. Patent No. 5,763,223, including soluble forms thereof.

"TWEAK" is TNF-weak effector of apoptosis, a type II transmembrane polypeptide in the TNF family described, inter alia, in Chicheportiche et al., J. Biol. Chem., 272(51):32401, 1997, including soluble forms thereof. "TWEAK-R" is the "TWEAK receptor," which is described, inter alia, in U.S. Serial Numbers 60/172,878 and 60/203,347 and Feng et al., Am. J. Pathol. 156(4):1253, 2000, including soluble forms thereof. TWEAK-R/Fc is a TWEAK receptor-Fc fusion polypeptide.

"VEGF" is vascular endothelial growth factor, also known as VPF or vascular permeability factor.

B. ADAM Polypeptides and ADAM Disintegrin Domain Polypeptides

At least thirty ADAMs have been described. Table 1 provides reference information for selected human ADAMs.

ADAM disintegrin domains show sequence homology to the snake venom disintegrins, and are characterized by a framework of cysteines. For example, a typical disintegrin sequence comprises a framework such as:

$CDCGX_{3-5}CX_{3-6}CCX_{2-4}CX_7CX_{4-6}CCX_{2-4}CX_8CX_{5-7}CX_{3-5}C$ (SEQ ID NO:20)

The sequences of several ADAM disintegrin domains are shown in Table 2 and in the Sequence Listing.

The present invention encompasses the use of various forms of ADAM disintegrin domains that retain at least one activity selected from the group consisting of integrin binding activity, inhibition of endothelial cell migration, and inhibition of angiogenesis. The term "ADAM disintegrin domain polypeptide" is intended to encompass polypeptides containing all or part of a native ADAM disintegrin domain, with or without other ADAM domains (such as the cysteine-rich region), as well as related forms including, but not limited to: (a) fragments, (b) variants, (c) derivatives, (d) fusion polypeptides, and (e) multimeric forms (multimers). The ability of these related forms to inhibit integrin binding, endothelial cell migration, and/or inhibition of angiogenesis may be determined in vitro or in vivo by using methods such as those exemplified below or by using other assays known in the art.

**Table 1**
Selected Members of the ADAM Family

| ADAM | Other Names | GenBank Accession Number (Human) | Published Description |
|---|---|---|---|
| ADAM-8 | MS2, CD156 | D26579 | Genomics 41(1):56, 1997 |
| ADAM-9 | MDC9, meltrin gamma | U41766 | J. Cell. Biol. 132(4):717, 1996 |
| ADAM-10 | MADM, kuzbanian, reprolysin | AF009615 | J. Biol. Chem. 272(39):24588, 1997 |
| ADAM-15 | Metargidin, MDC15 | U46005 | J. Biol. Chem. 271(9):4593, 1996 |
| ADAM-17 | TACE, cSVP | U86755 | WO 96/41624 |
| ADAM-20 | SVPH1-26 | AF029899 | WO 99/23228 |
| ADAM-21 | SVPH1-8 | AF029900 | WO 99/36549 |
| ADAM-22 | SVPH3-13, MDC2 | AB009671 | WO 99/41388 |
| ADAM-23 | SVPH3-17, MDC3 | AB009672 | WO 99/41388 |
| ADAM-29 | SVPH1 | AF171929 | Biochem. Biophys. Res. Commun. 263:810. 1999 |

The term "variant" includes polypeptides that are substantially homologous to native ADAM disintegrin domains, but which have an amino acid sequence different from that of a native ADAM disintegrin domain because of one or more deletions, insertions or substitutions. Particular embodiments include, but are not limited to, ADAM disintegrin domain polypeptides that comprise from one to ten deletions, insertions or substitutions of amino acid residues, when compared to a native ADAM disintegrin domain sequence. Included as variants of ADAM disintegrin domain polypeptides are those variants that are naturally occurring, such as allelic forms and alternatively spliced forms, as well as variants that have been constructed by modifying the amino acid sequence of a ADAM disintegrin domain polypeptide or the nucleotide sequence of a nucleic acid encoding a ADAM disintegrin domain polypeptide.

Generally, substitutions for one or more amino acids present in the native polypeptide should be made conservatively. Examples of conservative substitutions include substitution of amino acids outside of the active domain(s), and substitution of amino acids that do not alter the secondary and/or tertiary structure of the ADAM disintegrin domain. Additional examples include substituting one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another, or substitutions of one polar residue for another, such as between Lys and Arg; Glu and Asp; or Gln and Asn, or substitutions of one aromatic residue for another, such as Phe, Trp, or Tyr for one another. Other such conservative substitutions, for example, substitutions of entire regions having similar hydrophobicity characteristics, are known in the art.

In some preferred embodiments the ADAM disintegrin domain variant is at least about 70% identical in amino acid sequence to the amino acid sequence of a native ADAM disintegrin domain; in some preferred embodiments the ADAM disintegrin domain variant is at least about 80% identical in amino acid sequence to the amino acid sequence of a native ADAM disintegrin domain. In some more preferred embodiments the ADAM disintegrin domain variant is at least about 90% identical in amino acid sequence to the amino acid sequence of a native ADAM disintegrin domain; in some more preferred embodiments the ADAM disintegrin domain variant is at least about 95% identical in amino acid sequence to the amino acid sequence of a native ADAM disintegrin domain. In some most preferred embodiments the ADAM disintegrin domain variant is at least about 98% identical in amino acid sequence to the amino acid sequence of a native ADAM disintegrin domain; in some most preferred embodiments the ADAM disintegrin domain variant is at least about 99% identical in amino acid sequence to the amino acid sequence of a native ADAM disintegrin domain.

Percent identity, in the case of both polypeptides and nucleic acids, may be determined by visual inspection. Percent identity may be determined using the alignment method of Needleman and Wunsch (J. Mol. Biol. 48:443, 1970) as revised by Smith and Waterman (Adv. Appl. Math 2:482, 1981. Preferably, percent identity is determined by using a computer program, for example, the GAP computer program version 10.x available from the Genetics Computer Group (GCG; Madison, WI, see also Devereux et al., *Nucl. Acids Res.* 12:387, 1984). The preferred default parameters for the GAP program include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-

identities) for nucleotides, and the weighted comparison matrix of Gribskov and Burgess, *Nucl. Acids Res. 14*:6745, 1986, as described by Schwartz and Dayhoff, eds., *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, pp. 353-358, 1979 for amino acids; (2) a penalty of 30 (amino acids) or 50 (nucleotides) for each gap and an additional 1 (amino acids) or 3 (nucleotides) penalty for each symbol in each gap; (3) no penalty for end gaps; and (4) no maximum penalty for long gaps. Other programs used by one skilled in the art of sequence comparison may also be used. For fragments of ADAM disintegrin domains, the percent identity is calculated based on that portion of ADAM disintegrin domain that is present in the fragment.

When a deletion or insertion strategy is adopted, the potential effect of the deletion or insertion on biological activity (such as integrin binding activity, inhibition of endothelial cell migration, or inhibition of angiogenesis) must be considered. Subunits of the inventive polypeptides may be constructed by deleting terminal or internal residues or sequences. Additional guidance as to the types of mutations that can be made is provided by a comparison of the sequence of ADAM disintegrin domain polypeptides to polypeptides that have similar structures, as well as by performing structural analysis of the inventive polypeptides.

The term "variant" also includes ADAM disintegrin domain polypeptides that are encoded by nucleic acids capable of hybridizing under moderately stringent conditions (e.g., prewashing solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0) and hybridization conditions of 50°C, 5 X SSC, overnight) or higher stringency conditions to DNA sequences encoding ADAM disintegrin domain polypeptides, and which encode polypeptides that retain at least one activity selected from the group consisting of integrin binding activity, inhibition of endothelial cell migration, and inhibition of angiogenesis. The skilled artisan can determine additional combinations of salt and temperature that constitute moderate hybridization stringency. Conditions of higher stringency include higher temperatures for hybridization and post-hybridization washes, and/or lower salt concentration.

Mutations can be introduced into nucleic acids by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes a variant having the desired amino acid insertion, substitution, or deletion. Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered gene having particular codons altered according to the substitution, deletion, or insertion required. The well known polymerase chain reaction (PCR) procedure also may be employed to generate and amplify a DNA sequence encoding a desired polypeptide or fragment thereof. Oligonucleotides that define the desired termini of the DNA fragment are employed as 5' and 3' primers. The oligonucleotides may additionally contain recognition sites for restriction endonucleases to facilitate insertion of the amplified DNA fragment into an expression vector.

The present invention further encompasses the use of ADAM disintegrin domain polypeptides with or without associated native-pattern glycosylation. ADAM disintegrin domain expressed in yeast or mammalian expression systems (e.g., COS-1 or COS-7 cells) may be similar to or significantly

different from a native ADAM disintegrin domain polypeptide in molecular weight and glycosylation pattern, depending upon the choice of expression system. Expression of ADAM disintegrin domain polypeptides in bacterial expression systems, such as *E. coli*, provides non-glycosylated molecules. Different host cells may also process polypeptides differentially, resulting in heterogeneous mixtures of polypeptides with variable N- or C-termini.

The primary amino acid structure of ADAM disintegrin domain polypeptides may be modified to create derivatives by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups and the like. Covalent derivatives of ADAM disintegrin domain polypeptides may be prepared by linking particular functional groups to ADAM disintegrin domain amino acid side chains or at the N-terminus or C-terminus of a ADAM disintegrin domain polypeptide.

Fusion polypeptides of ADAM disintegrin domains that are useful in practicing the invention include covalent or aggregative conjugates of ADAMdis or its fragments with other polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions. One class of fusion polypeptides are discussed below in connection with ADAM disintegrin oligomers. As another example, a fusion polypeptide may comprise a signal peptide (which is also variously referred to as a signal sequence, signal, leader peptide, leader sequence, or leader) at the N-terminal region or C-terminal region of an ADAM disintegrin domain polypeptide which co-translationally or post-translationally directs transfer of the polypeptide from its site of synthesis to a site inside or outside of the cell membrane or cell wall. It is particularly advantageous to fuse a signal peptide that promotes extracellular secretion to the N-terminus of a soluble ADAMdis polypeptide. In this case, the signal peptide is typically cleaved upon secretion of the soluble polypeptide from the cell.

Secreted soluble polypeptides may be identified (and distinguished from its non-soluble membrane-bound counterparts) by separating intact cells which express the desired polypeptide from the culture medium, e.g., by centrifugation, and assaying the medium (supernatant) for the presence of the desired polypeptide. The presence of the desired polypeptide in the medium indicates that the polypeptide was secreted from the cells and thus is a soluble form of the polypeptide. Soluble polypeptides may be prepared by any of a number of conventional techniques. A DNA sequence encoding a desired soluble polypeptide may be subcloned into an expression vector for production of the polypeptide, or the desired encoding DNA fragment may be chemically synthesized.

Soluble ADAM disintegrin domain polypeptides comprise all or part of the ADAM disintegrin domain, with or without additional segments from the extracellular portion of the ADAM (such as the cysteine-rich region) but generally lack a transmembrane domain that would cause retention of the polypeptide at the cell surface. Soluble polypeptides may include part of the transmembrane domain or all or part of the cytoplasmic domain as long as the polypeptide is secreted from the cell in which it is produced. Examples of soluble ADAM disintegrin domain polypeptides are provided in the examples. In some preferred embodiments of the present invention, a multimeric form of a soluble ADAM disintegrin domain polypeptide is used to inhibit integrin binding to ligands

and, hence, integrin biological activity. In some most preferred embodiments the soluble ADAM disintegrin domain polypeptide is used to inhibit endothelial cell migration and/or inhibit angiogenesis. These inhibitory activities may include both integrin-mediated and integrin-independent mechanisms.

ADAM disintegrin domain multimers are covalently-linked or non-covalently-linked multimers, including dimers, trimers, and higher multimers. Oligomers may be linked by disulfide bonds formed between cysteine residues on different ADAM disintegrin domain polypeptides. One embodiment of the invention is directed to multimers comprising multiple ADAM disintegrin domain polypeptides joined via covalent or non-covalent interactions between peptide moieties fused to the ADAM disintegrin domain polypeptides. Such peptides may be peptide linkers (spacers), or peptides that have the property of promoting multimerization. Leucine zippers and certain polypeptides derived from antibodies are among the peptides that can promote multimerization of ADAM disintegrin domain polypeptides attached thereto, as described in more detail below. In particular embodiments, the multimers comprise from two to four ADAM disintegrin domain polypeptides.

In some embodiments, a ADAM disintegrin domain multimer is prepared using polypeptides derived from immunoglobulins. Preparation of fusion proteins comprising certain heterologous polypeptides fused to various portions of antibody-derived polypeptides (including the Fc domain) has been described, e.g., by Ashkenazi et al. (Proc. Natl. Acad. Sci. USA 88:10535, 1991); Byrn et al. (Nature 344:677, 1990); and Hollenbaugh and Aruffo ("Construction of Immunoglobulin Fusion Proteins", in Current Protocols in Immunology, Suppl. 4, pages 10.19.1-10.19.11, 1992).

A preferred embodiment of the present invention is directed to an ADAM disintegrin domain (ADAMdis) dimer comprising two fusion polypeptides created by fusing an ADAM disintegrin domain to an Fc polypeptide. A gene fusion encoding the ADAMdis-Fc fusion polypeptide is inserted into an appropriate expression vector. ADAMdis-Fc fusion polypeptides are expressed in host cells transformed with the recombinant expression vector, and allowed to assemble much like antibody molecules, whereupon interchain disulfide bonds form between the Fc moieties to yield divalent soluble ADAMdis polypeptides. The term "Fc polypeptide" as used herein includes native and mutein forms of polypeptides derived from the Fc region of an antibody. Truncated forms of such polypeptides containing the hinge region that promotes dimerization are also included.

One suitable Fc polypeptide, described in PCT application WO 93/10151, is a single chain polypeptide extending from the N-terminal hinge region to the native C-terminus of the Fc region of a human IgG1 antibody. Another useful Fc polypeptide is the Fc mutein described in U.S. Patent 5,457,035 and by Baum et al., EMBO J. 13:3992, 1994. The amino acid sequence of this mutein is identical to that of the native Fc sequence presented in WO 93/10151, except that amino acid 19 has been changed from Leu to Ala, amino acid 20 has been changed from Leu to Glu, and amino acid 22 has been changed from Gly to Ala. The mutein exhibits reduced affinity for Fc receptors. Fusion polypeptides comprising Fc moieties, and multimers formed therefrom, offer an advantage of facile purification by affinity chromatography over Protein A or Protein G columns, and Fc fusion

polypeptides may provide a longer in vivo half life, which is useful in therapeutic applications, than unmodified polypeptides.

In other embodiments, a soluble ADAM disintegrin domain polypeptide may be substituted for the variable portion of an antibody heavy or light chain. If fusion proteins are made with both heavy and light chains of an antibody, it is possible to form an ADAM disintegrin domain multimer with as many as four soluble ADAM disintegrin domain polypeptides.

Alternatively, the ADAM disintegrin domain multimer is a fusion polypeptide comprising multiple ADAM disintegrin domain polypeptides, with or without peptide linkers (spacers), or peptides that have the property of promoting multimerization.. Among the suitable peptide linkers are those described in U.S. Patents 4,751,180 and 4,935,233. A DNA sequence encoding a desired peptide linker may be inserted between, and in the same reading frame as, the DNA sequences encoding ADAMdis, using conventional techniques known in the art. For example, a chemically synthesized oligonucleotide encoding the linker may be ligated between sequences encoding ADAMdis. In particular embodiments, a fusion protein comprises from two to four ADAM disintegrin domain polypeptides, separated by peptide linkers.

Another method for preparing ADAM disintegrin domain multimers involves use of a leucine zipper domain. Leucine zipper domains are peptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., Science 240:1759, 1988), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble oligomeric proteins are described in PCT application WO 94/10308, and the leucine zipper derived from lung surfactant protein D (SPD) described in Hoppe et al. FEBS Lett. 344:191, 1994. The use of a modified leucine zipper that allows for stable trimerization of a heterologous protein fused thereto is described in Fanslow et al., Semin. Immunol. 6:267, 1994. Recombinant fusion polypeptides comprising an ADAM disintegrin domain polypeptide fused to a leucine zipper peptide are expressed in suitable host cells, and the ADAM disintegrin domain multimer that forms is recovered from the culture supernatant.

C. Recombinant Production of ADAM Disintegrin Domain Polypeptides

The ADAM disintegrin domain polypeptides used in the present invention may be prepared using a recombinant expression system. Host cells transformed with a recombinant expression vector encoding the ADAM disintegrin domain polypeptide are cultured under conditions that promote expression of ADAM disintegrin domain and the ADAM disintegrin domain is recovered. ADAM disintegrin domain polypeptides can also be produced in transgenic plants or animals.

Any suitable expression system may be employed. Recombinant expression vectors include DNA encoding an ADAM disintegrin domain polypeptide operably linked to suitable transcriptional

and translational regulatory nucleotide sequences, such as those derived from a mammalian, microbial, viral, or insect gene. Nucleotide sequences are operably linked when the regulatory sequence functionally relates to the ADAM disintegrin domain DNA sequence. Thus, a promoter nucleotide sequence is operably linked to an ADAM disintegrin domain DNA sequence if the promoter nucleotide sequence controls the transcription of the ADAM disintegrin domain DNA sequence. Examples of regulatory sequences include transcriptional promoters, operators, or enhancers, an mRNA ribosomal binding site, and appropriate sequences which control transcription and translation initiation and termination. A sequence encoding an appropriate signal peptide (native or heterologous) can be incorporated into expression vectors. A DNA sequence for a signal peptide (secretory leader) may be fused in frame to the ADAM disintegrin domain sequence so that the ADAM disintegrin domain polypeptide is initially translated as a fusion protein comprising the signal peptide. A signal peptide that is functional in the intended host cells promotes extracellular secretion of the ADAM disintegrin domain polypeptide. The signal peptide is cleaved from the ADAM disintegrin domain polypeptide upon secretion from the cell. Suitable host cells for expression of ADAM disintegrin domain polypeptides include prokaryotes, yeast and higher eukaryotic cells, including insect and mammalian cells. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, insect, and mammalian cellular hosts are known in the art.

Using the techniques of recombinant DNA including mutagenesis and the polymerase chain reaction (PCR), the skilled artisan can produce DNA sequences that encode ADAM disintegrin domain polypeptides comprising various additions or substitutions of amino acid residues or sequences, or deletions of terminal or internal residues or sequences, including ADAM disintegrin domain fragments, variants, derivatives, multimers, and fusion polypeptides.

The procedures for purifying expressed ADAM disintegrin domain polypeptides will vary according to the host system employed, and whether or not the recombinant polypeptide is secreted. ADAM disintegrin domain polypeptides may be purified using methods known in the art, including one or more concentration, salting-out, ion exchange, hydrophobic interaction, affinity purification, HPLC, or size exclusion chromatography steps. Fusion polypeptides comprising Fc moieties (and multimers formed therefrom) offer the advantage of facile purification by affinity chromatography over Protein A or Protein G columns.

D. Therapeutic Methods

The disclosed methods may be used to inhibit integrin binding and integrin biological activity, and to inhibit endothelial-cell migration, and/or angiogenesis in a mammal in need of such treatment. The treatment is advantageously administered in order to prevent the onset or the recurrence of a disease or condition mediated by an integrin, or to treat a mammal that has a disease or condition mediated by an integrin.

Examples of the therapeutic uses of ADAM disintegrin domain polypeptides and compositions thereof include the treatment of individuals afflicted with conditions mediated by

angiogenesis such as ocular disorders, dermatological disorders, and malignant or metastatic conditions, inflammatory diseases, osteoporosis and other conditions mediated by accelerated bone resorption, restenosis, inappropriate platelet activation, recruitment, or aggregation, thrombosis, or a condition requiring tissue repair or wound healing.

Among the ocular disorders that can be treated according to the present invention are eye diseases characterized by ocular neovascularization including, but not limited to, diabetic retinopathy (a major complication of diabetes), retinopathy of prematurity (this devastating eye condition, that frequently leads to chronic vision problems and carries a high risk of blindness, is a severe complication during the care of premature infants), neovascular glaucoma, retinoblastoma, retrolental fibroplasia, rubeosis, uveitis, macular degeneration, and corneal graft neovascularization. Other eye inflammatory diseases, ocular tumors, and diseases associated with choroidal or iris neovascularization can also be treated according to the present invention.

The present invention can also be used to treat malignant and metastatic conditions such as solid tumors. Solid tumors include both primary and metastatic sarcomas and carcinomas.

The present invention can also be used to treat inflammatory diseases including, but not limited to, arthritis, rheumatism, inflammatory bowel disease, and psoriasis.

Among the conditions mediated by inappropriate platelet activation, recruitment, aggregation, or thrombosis that can be treated according to the present invention are coronary artery disease or injury, myocardial infarction or injury following myocardial infarction, stroke, unstable angina, atherosclerosis, arteriosclerosis, preeclampsia, embolism, platelet-associated ischemic disorders including lung ischemia, coronary ischemia, and cerebral ischemia, restenosis following percutaneous coronary intervention including angioplasty, atherectomy, stent placement, and bypass surgery, thrombotic disorders including coronary artery thrombosis, cerebral artery thrombosis, intracardiac thrombosis, peripheral artery thrombosis, venous thrombosis, thrombosis and coagulopathies associated with exposure to a foreign or injured tissue surface, and reocclusion following thrombosis, deep venous thrombosis (DVT), pulmonary embolism (PE), transient ischemic attacks (TIAs), and another conditions where vascular occlusion is a common underlying feature. In some embodiments the methods according to the invention are used in individuals at high risk for thrombus formation or reformation, advanced coronary artery disease, or for occlusion, reocclusion, stenosis and/or restenosis of blood vessels, or stroke. In some embodiments the methods according to the invention are used in combination with angioplasty procedures, such as balloon angioplasty, laser angioplasty, coronary atherectomy or similar techniques, carotid endarterectomy, anastomosis of vascular grafts, surgery having a high risk of thrombus formation (i.e., coronary bypass surgery, insertion of a prosthetic valve or vessel and the like), atherectomy, stent placement, placement of a chronic cardiovascular device such as an in-dwelling catheter or prosthetic valve or vessel, organ transplantation, or bypass surgery.

Other diseases and conditions that can be treated according to the present invention include benign tumors and preneoplastic conditions, myocardial angiogenesis, hemophilic joints, scleroderma,

vascular adhesions, asthma and allergy, eczema and dermatitis, graft versus host disease, sepsis, adult respirator distress syndrome, telangiectasia, and wound granulation.

The methods according to the present invention can be tested in in vivo animal models for the desired prophylactic or therapeutic activity, as well as to determine the optimal therapeutic dosage, prior to administration to humans.

The amount of a particular ADAM disintegrin domain polypeptide that will be effective in a particular method of treatment depends upon age, type and severity of the condition to be treated, body weight, desired duration of treatment, method of administration, and other parameters. Effective dosages are determined by a physician or other qualified medical professional. Typical effective dosages are about 0.01 mg/kg to about 100 mg/kg body weight. In some preferred embodiments the dosage is about 0.1-50 mg/kg; in some preferred embodiments the dosage is about 0.5-10 mg/kg. The dosage for local administration is typically lower than for systemic administration. In some embodiments a single administration is sufficient; in some embodiments the ADAM disintegrin domain is administered as multiple doses over one or more days.

The ADAM disintegrin domain polypeptides are typically administered in the form of a pharmaceutical composition comprising one or more pharmacologically acceptable carriers. Pharmaceutically acceptable carriers include diluents, fillers, adjuvants, excipients, and vehicles which are pharmaceutically acceptable for the route of administration, and may be aqueous or oleaginous suspensions formulated using suitable dispersing, wetting, and suspending agents.

Pharmaceutically acceptable carriers are generally sterile and free of pyrogenic agents, and may include water, oils, solvents, salts, sugars and other carbohydrates, emulsifying agents, buffering agents, antimicrobial agents, and chelating agents. The particular pharmaceutically acceptable carrier and the ratio of active compound to carrier are determined by the solubility and chemical properties of the composition, the mode of administration, and standard pharmaceutical practice.

The ADAM disintegrin domain polypeptides are administered to the patient in a manner appropriate to the indication. Thus, for example, ADAM disintegrin domain polypeptides, or pharmaceutical compositions thereof, may be administered by intravenous, transdermal, intradermal, intraperitoneal, intramuscular, intranasal, epidural, oral, topical, subcutaneous, intracavity, sustained release from implants, peristaltic routes, or by any other suitable technique. Parenteral administration is preferred.

In certain embodiments of the claimed invention, the treatment further comprises treating the mammal with one or more additional therapeutic agents. The additional therapeutic agent(s) may be administered prior to, concurrently with, or following the administration of the ADAM disintegrin domain polypeptide. The use of more than one therapeutic agent is particularly advantageous when the mammal that is being treated has a solid tumor. In some embodiments of the claimed invention, the treatment further comprises treating the mammal with radiation. Radiation, including brachytherapy and teletherapy, may be administered prior to, concurrently with, or following the administration of the ADAM disintegrin domain polypeptide and/or additional therapeutic agent(s).

In some preferred embodiments the method includes the administration of, in addition to an ADAM disintegrin domain polypeptide, one or more therapeutics selected from the group consisting of alkylating agents, antimetabolites, vinca alkaloids and other plant-derived chemotherapeutics, antitumor antibiotics, antitumor enzymes, topoisomerase inhibitors, platinum analogs, adrenocortical suppressants, hormones and antihormones, antibodies, immunotherapeutics, radiotherapeutics, and biological response modifiers.

In some preferred embodiments the method includes administration of, in addition to an ADAM disintegrin domain polypeptide, one or more therapeutics selected from the group consisting of cisplatin, cyclophosphamide, mechloretamine, melphalan, bleomycin, carboplatin, fluorouracil, 5-fluorodeoxyuridine, methotrexate, taxol, asparaginase, vincristine, and vinblastine, lymphokines and cytokines such as interleukins, interferons (alpha., beta. or delta.) and TNF, chlorambucil, busulfan, carmustine, lomustine, semustine, streptozocin, dacarbazine, cytarabine, mercaptopurine, thioguanine, vindesine, etoposide, teniposide, dactinomycin, daunorubicin, doxorubicin, bleomycin, plicamycin, mitomycin, L-asparaginase, hydroxyurea, methylhydrazine, mitotane, tamoxifen, fluoxymesterone, IL-8 inhibitors, angiostatin, endostatin, kringle 5, angiopoietin-2 or other antagonists of angiopoietin-1, antagonists of platelet-activating factor, antagonists of basic fibroblast growth factor, and COX-2 inhibitors.

In some preferred embodiments the method includes administration of, in addition to an ADAM disintegrin domain polypeptide, one or more therapeutic polypeptides, including soluble forms thereof, selected from the group consisting of Flt3 ligand, CD40 ligand, interleukin-2, interleukin-12, 4-1BB ligand, anti-4-1BB antibodies, TRAIL, TNF antagonists and TNF receptor antagonists including TNFR/Fc, Tek antagonists, TWEAK antagonists and TWEAK-R antagonists including TWEAK-R/Fc, VEGF antagonists including anti-VEGF antibodies, VEGF receptor (including VEGF-R1 and VEGF-R2, also known as Flt1 and Flk1 or KDR) antagonists, CD148 (also referred to as DEP-1, ECRTP, and PTPRJ, see Takahashi et al., J. Am. Soc. Nephrol. 10:2135-45, 1999; and PCT Publication No. WO 00/15258, 23 March 2000) binding proteins, and nectin-3 antagonists.

In some preferred embodiments the ADAM disintegrin domain polypeptides of the invention are used as a component of, or in combination with, "metronomic therapy," such as that described by Browder et al. and Klement et al. (Cancer Research 60:1878, 2000; J. Clin. Invest. 105(8):R15, 2000; see also Barinaga, Science 288:245, 2000).

As used herein, the terms "therapy," "therapeutic," "treat," and "treatment" generally include prophylaxis, i.e. prevention, in addition to therapy or treatment for an extant disease or condition. The methods of the present invention may be used as a first line treatment, for the treatment of residual disease following primary therapy, or as an adjunct to other therapies. Methods of measuring biological effectiveness are known in the art and are illustrated in the Examples below.

## EXAMPLES

The following examples are intended to illustrate particular embodiments and not to limit the scope of the invention.

### EXAMPLE 1
### ADAM Disintegrin Domain Polypeptides

This example describes one method for the recombinant production of ADAM disintegrin domain polypeptides.

Expression cassettes encoding an IgKappa leader sequence, ADAM disintegrin domain, and C-terminal Fc region were constructed in bacterial plasmids then transferred into eukaryotic expression vectors (pDC409, EMBO J. 10:2821, 1991, or another mammalian expression vector). The coding regions of the various constructs are summarized in Table 2. In addition to the disintegrin domain, these constructs encode additional portions of the extracellular portion of the ADAM (e.g., cysteine-rich region and EGF-like domain).

The expression vectors were transfected into COS-1, CV-1/EBNA, or 293/EBNA cells. Two days after transfection the cells were $^{35}S$ labeled for four hours. Supernatants and total cell lysates were prepared and aliquots were immunoprecipitated using protein A-sepharose beads to capture the Fc tagged polypeptides. $^{35}S$ labeled ADAM disintegrin-Fc polypeptides were run on 8-16% reducing gels and detected via autoradiography.

The cell type that produced the most soluble protein in the supernatant was used in a large scale (T-175 format, 20 flasks) transient transfection, and approximately one liter of supernatant was harvested after one week. ADAM disintegrin-Fc polypeptides were purified from the supernatants using affinity chromatography (protein A column). The polypeptides were characterized by determining the N-terminal amino acid sequence, amino acid composition, and protein integrity (SDS-PAGE under reducing and non-reducing conditions) before the polypeptides were used in FACS, immunoprecipitations, and biological assays such as those described below.

**Table 2**

ADAM Disintegrin Domain Polypeptide Constructs

| Construct | SEQ ID NOs: DNA/polypeptide | IgK Leader[1,2] | ADAM disintegrin[1,3] (dis Framework)[1,4] | Fc Region[1] |
|---|---|---|---|---|
| ADAM-8dis-Fc | 1/2 | 1-20 | 23-264 (34-91) | 267-494 |
| ADAM-9dis-Fc | 3/4 | 1-20 | 23-303 (34-92) | 306-533 |
| ADAM-10dis-Fc | 5/6 | 1-20 | 23-235 (34-99) | 238-465 |
| ADAM-15dis-Fc | 7/8 | 1-20 | 23-292 (34-92) | 295-522 |
| ADAM-17dis-Fc | 9/10 | 1-20 | 23-216 (34-93) | 219-446 |
| ADAM-20dis-Fc | 11/12 | 1-20 | 23-305 (34-91) | 308-535 |
| ADAM-21dis-Fc | 13/14 | 1-20 | 23-293 (34-91) | 296-523 |
| ADAM-22dis-Fc | 15/16 | 1-20 | 23-312 (34-92) | 315-542 |
| ADAM-23dis-Fc | 17/18 | 1-20 | 23-310 (34-91) | 313-540 |
| ADAM-29dis-Fc | 21/22 | 1-20 | 23-298 (34-91) | 301-528 |

[1] residues in the polypeptide sequence
[2] the predicted cleavage site is after residue 20
[3] segment of the construct that includes ADAMdis, but may also contain additional ADAM sequences
[4] disintegrin framework, e.g., SEQ ID NO:20

## EXAMPLE 2
## Binding of ADAM Disintegrin Domain Polypeptides to Cells

A. Binding to Endothelial cells

This example describes a flow cytometric integrin mAb based binding inhibition assay, which is used to show binding of ADAM disintegrin-Fc polypeptides to integrins expressed on the surface of endothelial cells. Human endothelial cells express $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\beta_1$, $\beta_4$, $\alpha_1$, $\alpha_2$, $\alpha_3$, $\alpha_4$, $\alpha_5$, and $\alpha_6$ integrins.

Primary human dermal microvascular endothelial cells (HMVEC-d) were maintained in supplemented endothelial growth medium (Clonetics Corporation, Walkersville, MD). The ADAM disintegrin-Fc polypeptides produced in Example 1 were shown to bind specifically to HMVEC-d.

Monoclonal antibodies specific for human integrins $\alpha_v\beta_3$ (LM609, anti CD51/61, Chemicon, Temecula, CA Brooks et al., Science 264:569, 1994), $\alpha_2\beta_1$ (BHA2.1 anti CD49b, Chemicon, Wang et al., Mol. Biol. of the Cell 9:865, 1998), $\alpha_5\beta_1$ (SAM-1 anti CD49e, Biodesign, A. te Velde et al., J. Immunol. 140:1548, 1988), $\alpha_3\beta_1$ (ASC-6 anti-CD49c, Chemicon, Pattaramalai et al., Exp. Cell. Res. 222: 281, 1996), $\alpha_4\beta_1$ (HP2/1 anti CD49d, Immunotech, Marseilles, France. Workshop of the 4th International Conference on Human Leukocyte Differentiation Antigens, Vienna Austria, 1989, workshop number p091), $\alpha_6\beta_1$ (GoH3 anti CD49f, Immunotech, Workshop 4th International Conference on Human Leukocyte Differentiation Antigens, workshop number p055), $\alpha_6\beta_4$ (439-9B anti CD104, Pharmingen, San Diego, CA., Schlossman et al., 1995 Leukocyte Typing V: White Cell Differntiation Antigens. Oxford University Press, New York), and $\alpha_v\beta_5$ (MAB 1961, Chemicon International, monoclonal anti-human integrin $\alpha_v\beta_5$ mAb, IgG1 isotype, inhibits $\alpha_v\beta_5$ mediated binding/adhesion to vitronectin/fibronectin; Weinaker, et al., J. Biol. Chem. 269:6940, 1994) were also shown to bind specifically to HMVEC-d. Each of these antibodies is known to specifically block binding of the indicated integrin to its ligands (e.g., fibronectin, vitronectin, fibrinogen). The ability of integrin mAbs to inhibit the binding of ADAM disintegrin-Fc polypeptides reveals which integrins the disintegrin domains bind and, indirectly, which integrin binding activities the disintegrin domains are able to antagonize. The ability of the antibodies to inhibit binding of the ADAM disintegrin-Fc polypeptides to endothelial cells was tested as described below.

Prior to performing binding studies, HMVEC-d were removed from culture vessels using trypsin-EDTA. The cells were washed in media containing serum and resuspended in binding medium which consisted of PBS containing 1 mM Ca2+, 1 mM Mg2+ and 0.5 mM Mn2+, 0.1% sodium azide, 10% Normal goat serum, 2% rabbit serum and 2% fetal bovine serum. Under these binding conditions, ADAM-8, -9, -10, -15, -17, -20, -21, -22, -23, and -29dis-Fc all bind to human endothelial cells.

One hundred microliters of cell suspension, containing 200,000 to 500,000 HMVEC-d, were added to 12x75mm plastic test tubes. Monoclonal antibodies specific for one of the integrins, or a control monoclonal antibody (CD29 or M15), were added to the cell suspensions at a concentration of 100 µg/ml (5-8 fold mass excess) 15 minutes prior to addition of disintegrin-Fc fusion proteins. ADAM disintegrin-Fc polypeptides and control Fc fusion polypeptides (P7.5II.Fc) were added, at various concentrations from 12.5 to 20 µg/ml, to the cell suspensions and incubated for 1 hour at 30° C. Unbound Fc polypeptides were washed away by centrifugation of cells in 2 mls of binding media. The washed cell pellets were resuspended in binding medium and then incubated at 30° C for 30 minutes with goat anti-human Fc-specific biotinylated antibody at a concentration of 2.5 µg/ml for 30 minutes. After centrifugation and washing of the cell pellets, the cells were resuspended in binding medium and bound anti-human Fc-biotin was detected by adding streptavidin-phycoerythrin conjugate to the cell suspension at a 1:1000 dilution (1 µg/ml) and incubating at 30° C for 30 minutes. The unbound streptavidin-phycoerythrin was washed away and the cells were resuspended in binding

medium containing propidum iodide. The level of fluorescent binding (disintegrin-Fc binding) was determined by flow cytometry.

The level of binding of each ADAM disintegrin-Fc polypeptide was determined in the presence of anti-integrin specific mAb and in the presence of control mAb. Both the intensity of binding (MFI) and the percentage of cells binding were determined. Percent inhibition was calculated using the formula [1 - (MFI control-MFI integrin mAb) / MFI control. The results of these studies are summarized in Table 3.

ADAM-15, -17, -20 and -22 disintegrin domain polypeptides bound to $\alpha_v\beta_3$; ADAM 23 disintegrin domain polypeptide bound to $\alpha_2\beta_1$; ADAM-15, -21, -22 and -23 disintegrin domain polypeptides bound to $\alpha_5\beta_1$; ADAM-10, -17, -22 and -23 disintegrin domain polypeptides bound to the $\alpha_6$ integrins; ADAM-10 and –15 disintegrin domain polypeptides bound to $\alpha_v\beta_5$. An excess of a non blocking $\alpha_v\beta_5$ antibody did significantly affect the binding of ADAM-10, -22, and –23 disintegrin polypeptides to endothelial cells, suggesting that these ADAMdis polypeptides interact with integrin sites other than or in addition to the ligand (e.g., fibronectin, vitronectin) binding site. Based upon results from a different type of assay, Cal et al. have reported that the ADAM-23 disintegrin domain interacts with the $\alpha_v\beta_3$ integrin through an RGD-independent mechanism (Molec. Biol. of the Cell 11:1457, 2000).

Binding experiments are repeated using other ADAM disintegrin domains and other monoclonal antibodies. ADAM disintegrin-Fc polypeptides that bind to selected integrins are further tested for the ability to disrupt integrin-ligand interactions and to modulate endothelial cell function, angiogenesis, and other biological activities in vitro and in vivo.

**Table 3**

Binding of ADAM Disintegrin-Fc Polypeptides to Integrins Expressed on Human Endothelial Cells

| ADAM | Integrin<br>Binding[1] (+ or – or ND, not done) and Percent (%) Binding[2] | | | | | | |
|---|---|---|---|---|---|---|---|
| | $\alpha_v\beta_3$ | $\alpha_2\beta_1$ | $\alpha_3\beta_1$ | $\alpha_4\beta_1$ | $\alpha_5\beta_1$ | $\alpha_6\beta_1$, $\alpha_6\beta_4$ | $\alpha_v\beta_5$ |
| ADAM-8 | ND | ND | – (<10) | – (<10) | ND | ND | – (<20) |
| ADAM-9 | – (<10) | – (<10) | – (<10) | – (<20) | – (<10) | – (<10) | – (<10) |
| ADAM-10 | – (<10) | – (<10) | – (<10) | – (<20) | – (<10) | + (48) | + (25) |
| ADAM-15 | + (60) | – (<10) | – (<10) | – (<20) | + (30) | – (<10) | + (25) |
| ADAM-17 | + (50) | – (<10) | – (<10) | – (<10) | – (<10) | + (69) | – (<10) |
| ADAM-20 | + (58) | – (<10) | – (<10) | – (<10) | – (<20) | – (<10) | – (<10) |
| ADAM-21 | – (<10) | – (<10) | – (<10) | – (<10) | + (54) | – (<10) | – (<10) |
| ADAM-22 | + (42) | – (<10) | – (<10) | – (<10) | + (36) | + (32) | – (<10) |
| ADAM-23 | – (<10) | + (22) | – (<10) | – (<10) | + (49) | + (31) | – (<10) |

[1]positive binding defined as >20% binding inhibition; normal background variation 5-10%, baseline positive approx. 2X over background

[2]percent inhibition of binding by ADAM-dis-Fc in the presence of 5-8 fold excess integrin mAb as compared to control mAb

B. Binding to Primary Human T-Cells

Primary human T-cells were purified from whole blood. These cells were used in FACS experiments to assess cell surface binding of purified ADAMdis-Fc polypeptides. ADAMdis-Fc binding was assessed with and without Con A (5 µg/ml) or immobilized OTK3 antibody (1 mg/ml, immobilized for 1 hour, 37°C) stimulation. ADAMdis-Fc polypeptides (20 µg/ml) were bound at either 4° C or 30° C in the presence of cations (Ca++, Mg++, Mn++, 0.5 mM each). Cell surface integrin expression was assessed using a panel of murine and rat anti-human integrin antibodies. $\alpha_v\beta_5$, $\alpha_1$, $\alpha_3$, $\alpha_4$, $\alpha_6$, $\beta_1$, and $\beta_7$ integrins were detected on the surface of these cells. ADAMdis-Fc polypeptides did not bind to primary human T-cells at 4° C. ADAM-8-, ADAM-9-, ADAM-15-, ADAM-20-, ADAM-21-, ADAM-22-, and ADAM-23-dis-Fc polypeptides did bind primary T-cells at 30° C with Con A stimulation. ADAMdis-Fc binding was not inhibited by a three-fold molar excess of antibodies to the integrins listed above.

C. Binding to Resting Platelets

Binding of ADAMdis-Fc polypeptides to citrated washed resting platelets was performed at 4°C or 30°C. Binding was analyzed by flow cytometry using a biotinylated-anti-human Fc specific antibody and streptavidin-PE. Resting platelets express the integrins CD41/CD61 and CD49e. ADAM-9dis-Fc and ADAM-8dis-Fc bound resting platelets at 30°C but not at 4°C. ADAM-9dis-Fc binding to resting platelets at 30°C was not inhibited by a ten-fold excess of CD41a mAb.

## EXAMPLE 3
### Activity of ADAM Disintegrin Domain Polypeptides In a Wound Closure Assay

A planar endothelial cell migration (wound closure) assay was used to quantitate the inhibition of angiogenesis by ADAM disintegrin-Fc polypeptides in vitro. In this assay, endothelial cell migration is measured as the rate of closure of a circular wound in a cultured cell monolayer. The rate of wound closure is linear, and is dynamically regulated by agents that stimulate and inhibit angiogenesis in vivo.

Primary human renal microvascular endothelial cells, HRMEC, were isolated, cultured, and used at the third passage after thawing, as described in Martin et al., In Vitro Cell Dev Biol 33:261, 1997. Replicate circular lesions, "wounds," (600-800 micron diameter) were generated in confluent HRMEC monolayers using a silicon-tipped drill press. At the time of wounding the medium (DMEM + 1% BSA) was supplemented with 20 ng/ml PMA (phorbol-12-myristate-13-acetate), a range of concentrations of ADAM disintegrin-Fc polypeptide, or combinations of PMA and ADAM disintegrin-Fc polypeptide. The residual wound area was measured as a function of time (0-12 hours) using a microscope and image analysis software (Bioquant, Nashville, TN). The relative migration rate was calculated for each agent and combination of agents by linear regression of residual wound

area plotted over time. The inhibition of PMA-induced endothelial migration by ADAM disintegrin-Fc polypeptides is shown in Table 4.

The effect of ADAM-dis-Fc polypeptides on EGF-induced migration was also determined. For these experiments EGF (epidermal growth factor, 40 ng/ml) was added to the medium, instead of PMA, at the time of wounding. The results are shown in Table 5.

**Table 4**

Effect of ADAM-15, -17, -20, and –23dis-Fc Polypeptides in PMA-Induced Endothelial Cell Wound Closure Migration Assay

| Expt. ID | No Addition | PMA 20 ng/ml | PMA + IgG | PMA + ADAM-15dis-Fc | PMA + ADAM-17dis-Fc | PMA + ADAM-20dis-Fc | PMA + ADAM-23dis-Fc |
|---|---|---|---|---|---|---|---|
| HL-H-142 15 µg/ml dis-Fc | 0.0436[1] (0.0016)[2] | 0.0655 (0.0004) | | | | 0.0499 (0.0009) 72%[3] | |
| HL-H-147 15 µg/ml dis-Fc | 0.0244 (0.0023) | 0.0424 (0.0002) | 0.0449 (0.0012) 0% | 0.0357 (0.0007) 37% | | | 0.0225 (0.0022) 100% |
| HL-H-153 15 µg/ml dis-Fc | 0.0253 0.00013 | 0.0460 (0.0022) | 0.0491 (0.006) 0% | | 0.0392 (0.0016) 33% | 0.0388 (0.005) 36% | 0.0317 (0.005) 70% |
| HL-H-154 15 µg/ml dis-Fc | 0.0119 (0.0012) | 0.0312 (0.0016) | | | 0.0283 (0.0008) 15% | 0.0160 (0.0017) 79% | |

[1] Slopes to average triplicate Y values and treat as a single data point in order to test whether the slopes are significantly different

[2] Data in parentheses is the +/- standard error of slopes

[3] Percent inhibition compared to migration rate observed in the presence of PMA

**Table 5**

Effect of ADAM-17, -20, and –23dis-Fc Polypeptides in EGF-Induced Endothelial Cell Wound Closure Migration Assay

| Expt. ID | No Addition | EGF 40 ng/ml | EGF + IgG | EGF + ADAM-17dis-Fc | EGF + ADAM-20dis-Fc | EGF + ADAM-23dis-Fc |
|---|---|---|---|---|---|---|
| HL-H-154 15 µg/ml dis-Fc | 0.0119 (0.0012) | 0.0378 (0.0061) | | 0.0242 (0.0029) 53% | 0.0172 (0.0031) 80% | 0.0310 (0.0036) 26% |
| HL-H-155 9 µg/ml dis-Fc | 0.0164 (0.0010) | 0.0468 (0.0059) | 0.0454 (0.0052) 5% | 0.0412 (0.0107) 18% | 0.0227 (0.0035) 79% | 0.0207 (0.0016) 86% |

[1] Slopes to average triplicate Y values and treat as a single data point in order to test whether the slopes are significantly different

[2] Data in parentheses is the +/- standard error of slopes

[3] Percent inhibition compared to migration rate observed in the presence of EGF alone

ADAM-20 and -23dis-Fc polypeptides showed the greatest inhibition of both EGF- and PMA-induced endothelial migration at 15 µg/ml. ADAM-15 and -17dis-Fc polypeptides were less

effective at inhibiting endothelial cell migration at 15 µg/ml. Hu IgG did not inhibite EGF- or PMA-induced endothelial cell migration in any of the experiments performed where it was included as a control Fc protein.

## EXAMPLE 4
### Activity of ADAM Disintegrin Domain Polypeptides In a Corneal Pocket Assay

A mouse corneal pocket assay is used to quantitate the inhibition of angiogenesis by ADAM disintegrin-Fc polypeptides in vivo. In this assay, agents to be tested for angiogenic or anti-angiogenic activity are immobilized in a slow release form in a hydron pellet, which is implanted into micropockets created in the corneal epithelium of anesthetized mice. Vascularization is measured as the appearance, density, and extent of vessel ingrowth from the vascularized corneal limbus into the normally avascular cornea.

Hydron pellets, as described in Kenyon et al., Invest Opthamol. & Visual Science 37:1625, 1996, incorporate sucralfate with bFGF (90 ng/pellet), bFGF and IgG (11 µg/pellet, control), or bFGF and a range of concentrations of ADAM disintegrin-Fc polypeptide. The pellets are surgically implanted into corneal stromal micropockets created by micro-dissection 1 mm medial to the lateral corneal limbus of 6-8 week old male C57BL mice. After five days, at the peak of neovascular response to bFGF, the corneas are photographed, using a Zeiss slit lamp, at an incipient angle of 35-50° from the polar axis in the meridian containing the pellet. Images are digitized and processed by subtractive color filters (Adobe Photoshop 4.0) to delineate established microvessels by hemoglobin content. Image analysis software (Bioquant, Nashville, TN) is used to calculate the fraction of the corneal image that is vascularized, the vessel density within the vascularized area, and the vessel density within the total cornea. The inhibition of bFGF-induced corneal angiogenesis, as a function of the dose of ADAM disintegrin-Fc polypeptide, is determined.

## EXAMPLE 5
### Inhibition of Neovascularization by ADAM Disintegrin Domain Polypeptides in a Murine Transplant Model

Survival of heterotopically transplanted cardiac tissue from one mouse donor to the ear skin of another genetically similar mouse requires adequate neovascularization by the transplanted heart and the surrounding tissue, to promote survival and energy for cardiac muscle function. Inadequate vasculature at the site of transplant causes excessive ischemia to the heart, tissue damage, and failure of the tissue to engraft. Agents that antagonize factors involved in endothelial cell migration and vessel formation can decrease angiogenesis at the site of transplant, thereby limiting graft tissue function and ultimately engraftment itself. A murine heterotopic cardiac isograft model is used to demonstrate the antagonistic effects of ADAM disintegrin-Fc polypeptides on neovascularization. Female BALB/c (≈12 weeks of age) recipients are given neonatal heart grafts from donor mice of the same strain. The donor heart tissue is grafted into the left ear pinnae of the recipient on day 0 and the

mice are divided into two groups. The control group receives human IgG (Hu IgG) while the other group receives ADAM disintegrin-Fc polypeptide, both intraperitoneally. The treatments are continued for five consecutive days. The functionality of the grafts is determined by monitoring visible pulsatile activity on days 7 and 14 post-engraftment. The inhibition of functional engraftment, as a function of the dose of ADAM disintegrin-Fc polypeptide, is determined. The histology of the transplanted hearts is examined is order to visualize the effects of ADAM disintegrin-Fc polypeptides on edema at the site of transplant and host and donor tissue vasculature (using, e.g., Factor VIII staining).

## EXAMPLE 6
### Treatment of Tumors With ADAM Disintegrin Domain Polypeptides

ADAM disintegrin-Fc polypeptides are tested in animal models of solid tumors. The effect of the ADAM disintegrin-Fc polypeptides is determined by measuring tumor frequency and tumor growth.

The biological activity of ADAM disintegrin-Fc polypeptides is also demonstrated in other in vitro, ex vivo, and in vivo assays known to the skilled artisan, such as calcium mobilization assays and assays to measure platelet activation, recruitment, or aggregation.

The relevant disclosures of publications cited herein are specifically incorporated by reference. The examples presented above are not intended to be exhaustive or to limit the scope of the invention. The skilled artisan will understand that variations and modifications and variations are possible in light of the above teachings, and such modifications and variations are intended to be within the scope of the invention.

# CLAIMS

We claim:

1. A method of antagonizing the binding of an integrin to its ligands comprising contacting a cell that expresses the integrin with an effective amount of an ADAM disintegrin domain polypeptide.

2. A method of antagonizing the binding of an integrin to its ligands in a mammal in need of such treatment comprising administering an effective amount of an ADAM disintegrin domain polypeptide.

3. The method of claim 2 wherein the mammal is afflicted with a condition selected from the group consisting of ocular disorders, malignant and metastatic conditions, inflammatory diseases, osteoporosis and other conditions mediated by accelerated bone resorption, restenosis, inappropriate platelet activation, recruitment, or aggregation, thrombosis, or a condition requiring tissue repair or wound healing.

4. A method of inhibiting angiogenesis in a mammal in need of such treatment, comprising administering to the mammal an inhibition-effective amount of an ADAM disintegrin domain polypeptide, wherein the disintegrin domain does not contain an RGD sequence.

5. The method of one of claims 1-4 wherein the ADAM disintegrin domain is in the form of a multimer.

6. The method of claim 5 wherein the multimer is a dimer or trimer.

7. The method of claim 5 wherein the multimer comprises an Fc polypeptide or a leucine zipper.

8. The method of one of claims 1-7 wherein the ADAM disintegrin domain is from a human ADAM.

9. The method of claim 8 wherein the ADAM disintegrin domain is from an ADAM selected from the group consisting of ADAM-8, ADAM-9, ADAM-10, ADAM-15, ADAM-17, ADAM-20, ADAM-21, ADAM-22, ADAM-23, and ADAM-29.

10. The method of claim 9 wherein the ADAM disintegrin domain is from ADAM-17, ADAM-20, or ADAM-23.

11. The method of one of claims 1-10 wherein the ADAM disintegrin domain polypeptide comprises an amino acid sequence selected from the group consisting of:

(a) amino acids 1-494 of SEQ ID NO:2, amino acids 23-264 of SEQ ID NO:2, amino acids 1-533 of SEQ ID NO:4, amino acids 23-303 of SEQ ID NO:4, amino acids 1-465 of SEQ ID NO:6, amino acids 23-235 of SEQ ID NO:6, amino acids 1-522 of SEQ ID NO:8, amino acids 23-292 of SEQ ID NO:8, amino acids 1-446 of SEQ ID NO:10, amino acids 23-216 of SEQ ID NO:10, amino acids 1-535 of SEQ ID NO:12, amino acids 23-305 of SEQ ID NO:12, amino acids 1-523 of SEQ ID NO:14, amino acids 23-293 of SEQ ID NO:14, amino acids 1-542 of SEQ ID NO:16, amino acids 23-312 of SEQ ID NO:16, amino acids 1-540 of SEQ ID NO:18, amino acids 23-310 of SEQ ID NO:18, amino acids 1-528 of SEQ ID NO:22, amino acids 23-298 of SEQ ID NO:22;

(b) fragments of the polypeptides of (a) wherein said fragments retain at least one ADAMdis activity;

(c) variants of the polypeptides of (a) or (b), wherein said variants retain at least one ADAMdis activity; and

(d) fusion polypeptides comprising the polypeptides of (a), (b), or (c), wherein said fusion polypeptides retain at least one ADAMdis activity.

12. The method of claim 11 wherein the ADAM disintegrin domain comprises an amino acid sequence selected from the group consisting of amino acids 34-91 of SEQ ID NO:2, 34-92 of SEQ ID NO:4, 34-99 of SEQ ID NO:6, 34-92 of SEQ ID NO:8, 34-93 of SEQ ID NO:10, 34-91 of SEQ ID NO:12, 34-91 of SEQ ID NO:14, 34-92 of SEQ ID NO:16, 34-91 of SEQ ID NO:18, or 34-91 of SEQ ID NO:22.

13. The method of one of claims 1-12 wherein the ADAM disintegrin domain polypeptide is a variant that is at least 70%, 80%, 90%, 95%, 98%, or 99% identical in amino acid sequence to a polypeptide selected from the group consisting of:

(a) amino acids 1-494 of SEQ ID NO:2, amino acids 23-264 of SEQ ID NO:2, amino acids 1-533 of SEQ ID NO:4, amino acids 23-303 of SEQ ID NO:4, amino acids 1-465 of SEQ ID NO:6, amino acids 23-235 of SEQ ID NO:6, amino acids 1-522 of SEQ ID NO:8, amino acids 23-292 of SEQ ID NO:8, amino acids 1-446 of SEQ ID NO:10, amino acids 23-216 of SEQ ID NO:10, amino acids 1-535 of SEQ ID NO:12, amino acids 23-305 of SEQ ID NO:12, amino acids 1-523 of SEQ ID NO:14, amino acids 23-293 of SEQ ID NO:14, amino acids 1-542 of SEQ ID NO:16, amino acids 23-312 of SEQ ID NO:16, amino acids 1-540 of SEQ ID NO:18, amino acids 23-310 of SEQ ID NO:18, amino acids 1-528 of SEQ ID NO:22, amino acids 23-298 of SEQ ID NO:22; and

(b) fragments of the polypeptides of (a),

wherein said variant polypeptide retains at least one ADAMdis activity.

14. The method of one of claims 1-10 wherein the ADAM disintegrin domain polypeptide is encoded by a nucleic acid comprising a sequence selected from the group consisting of:

(a) nucleotides 118-1599 of SEQ ID NO:1, nucleotides 184-909 of SEQ ID NO:1, nucleotides 46-1644 of SEQ ID NO:3, nucleotides 112-954 of SEQ ID NO:3, nucleotides 25-1419 of SEQ ID NO:5, nucleotides 91-729 of SEQ ID NO:5, nucleotides 41-1606 of SEQ ID NO:7, nucleotides 107-916 of SEQ ID NO:7, nucleotides 25-1362 of SEQ ID NO:9, nucleotides 91-672 of SEQ ID NO:9, nucleotides 25-1629 of SEQ ID NO:11, nucleotides 91-939 of SEQ ID NO:11, nucleotides 25-1593 of SEQ ID NO:13, nucleotides 91-903 of SEQ ID NO:13, nucleotides 25-1650 of SEQ ID NO:15, nucleotides 91-960 of SEQ ID NO:15, nucleotides 25-1644 of SEQ ID NO:17, nucleotides 91-954 of SEQ ID NO:17, nucleotides 118-1701 of SEQ ID NO:21, nucleotides 184-1011 of SEQ ID NO:21;

(b) sequences which, due to the degeneracy of the genetic code, encode a polypeptide encoded by a nucleic acid of (a); and

(c) sequences that hybridize under conditions of moderate or high stringency to a sequence of (a) or (b) and that encode a polypeptide that retains at least one ADAMdis activity.

15. The method of one of claim 11-14 wherein the ADAMdis activity is selected from the group consisting of integrin binding activity, inhibition of endothelial cell migration, and inhibition of angiogenesis.

16. The method of one of claims 1-15 wherein the ADAM disintegrin domain polypeptide has been produced by culturing a recombinant cell that encodes the ADAM disintegrin domain polypeptide under conditions permitting expression of the ADAM disintegrin domain polypeptide, and recovering the ADAM disintegrin domain polypeptide.

17. The method of one of claims 1-16 wherein the ADAM disintegrin domain polypeptide is present in a composition comprising a pharmaceutically acceptable carrier.

18. The method of claim 2 wherein the mammal has a disease or condition mediated by angiogenesis.

19. The method of claim 18 wherein the disease or condition is characterized by ocular neovascularization.

20. The method of claim 18 wherein the disease or condition is a solid tumor.

21. The method of one of claims 1-20 wherein the method further comprises treating the mammal with radiation.

22. The method of one of claims 1-21 wherein the method further comprises treating the mammal with a second therapeutic agent.

23. The method of claim 22 wherein the second therapeutic agent is selected from the group consisting of alkylating agents, antimetabolites, vinca alkaloids and other plant-derived chemotherapeutics, antitumor antibiotics, antitumor enzymes, topoisomerase inhibitors, platinum analogs, adrenocortical suppressants, hormones and antihormones, antibodies, immunotherapeutics, radiotherapeutics, and biological response modifiers.

24. The method of claim 22 wherein the second therapeutic agent is selected from the group consisting of cisplatin, cyclophosphamide, bleomycin, carboplatin, fluorouracil, 5-fluorouracil, 5-fluorodeoxyuridine, methotrexate, taxol, asparaginase, vincristine, vinblastine, mechloretamine, melphalan, 5-fluorodeoxyuridine, lymphokines and cytokines such as interleukins, interferons (alpha., beta. or delta.) and TNF, chlorambucil, busulfan, carmustine, lomustine, semustine, streptozocin, dacarbazine, cytarabine, mercaptopurine, thioguanine, vindesine, etoposide, teniposide, dactinomycin, daunorubicin, doxorubicin, bleomycin, plicamycin, mitomycin, L-asparaginase, hydroxyurea, methylhydrazine, mitotane, tamoxifen, fluoxymesterone, and COX-2 inhibitors.

25. The method of claim 22 wherein the second therapeutic agent is a polypeptide, including soluble forms thereof, selected from the group consisting of Flt3 ligand, CD40 ligand, interleukin-2, interleukin-12, 4-1BB ligand, anti-4-1BB antibodies, TRAIL, TNF antagonists and TNF receptor antagonists including TNFR/Fc, Tek antagonists, TWEAK antagonists and TWEAK-R antagonists including TWEAK-R/Fc, VEGF antagonists including anti-VEGF antibodies, VEGF receptor antagonists, CD148 binding proteins, and nectin-3 antagonists.

26. The method of claim 2 wherein the ADAM disintegrin domain is administered parenterally.

27. A method for inhibiting the biological activity of an integrin selected from the group consisting of $\alpha_v\beta_3$, $\alpha_2\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$, $\alpha_6\beta_4$, and $\alpha_v\beta_5$ comprising contacting the integrin with an inhibition-effective amount of an ADAM disintegrin domain polypeptide.

28. The method of claim 27 wherein the integrin is $\alpha_v\beta_3$ and wherein the ADAM disintegrin domain does not contain an RGD sequence.

29. The method of claim 28 wherein the ADAM is ADAM-17, ADAM-20, or ADAM-22.

30. The method of claim 27 wherein the integrin is $\alpha_2\beta_1$ and the ADAM is ADAM-23.

31. The method of claim 27 wherein the integrin is $\alpha_5\beta_1$ and the ADAM is ADAM-15 ADAM-21, ADAM-22, or ADAM-23.

32. The method of claim 27 wherein the integrin is $\alpha_6\beta_1$ or $\alpha_6\beta_4$ and the ADAM is ADAM-10, ADAM-17, ADAM-22, or ADAM-23.

33. The method of claim 27 wherein the integrin is $\alpha_v\beta_5$ and the ADAM is ADAM-10, ADAM-15, or ADAM-23.

34. A method for identifying a compound that modulates integrin biological activity comprising:

(a) combining a test compound with an integrin and an ADAM disintegrin domain polypeptide that binds to the integrin; and

(b) determining whether the test compound alters the binding of the ADAM disintegrin domain polypeptide to the integrin.

35. A method for identifying a compound that modulates the interaction between an integrin and an ADAM disintegrin domain comprising:

(a) combining a test compound with the integrin and an ADAM disintegrin domain polypeptide that binds to the integrin; and

(b) determining whether the test compound alters the binding of the ADAM disintegrin domain polypeptide to the integrin.

36. The method of claim 34 or 35 wherein the integrin is present on a cell surface.

37. The method of claim 36 wherein the cell is an endothelial cell.

38. The method of one of claims 34-37 wherein the integrin is selected from the group consisting of $\alpha_v\beta_3$, $\alpha_2\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$, $\alpha_6\beta_4$, and $\alpha_v\beta_5$.

39. The method of one of claims 34-38 wherein the integrin biological activity or integrin binding activity is at least partially inhibited.

40. A method for identifying a compound that inhibits endothelial cell migration and/or angiogenesis comprising:

(a) combining a test compound with endothelial cells and with an ADAM disintegrin domain polypeptide that binds to endothelial cells; and

(b) determining whether the test compound alters the binding of the ADAM disintegrin domain polypeptide to the endothelial cells.

41. The method of one of claims 34-40 wherein the ADAM disintegrin domain polypeptide comprises an ADAM disintegrin domain from ADAM-8, ADAM-9, ADAM-10, ADAM-15, ADAM-17, ADAM-20, ADAM-21, ADAM-22, ADAM-23, or ADAM-29.

42. The method of claim 41 wherein the ADAM disintegrin domain polypeptide comprises an ADAM disintegrin domain from ADAM-17, ADAM-20, or ADAM-23.

SEQUENCE LISTING

<110> Immunex Corporation
Fanslow, William C.
Poindexter, Kurt
Cerretti, Douglas P.
Black, Roy A.

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Ala Phe Trp Gly Pro Gly Gly Gln Ala Ala Glu Glu Ser Cys Phe Ser
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Tyr Asp Ile Leu Pro Gly Cys Lys Ala Ser Arg Tyr Arg Ala Asp Met
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Cys Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe
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Pro Asp Val Phe Ile Gln Asn Gly Tyr Pro Cys Gln Asn Asn Lys Ala
                 105                 110                 115

tat tgc tac aac ggc atg tgc cag tat tat gat gct caa tgt caa gtc   441
Tyr Cys Tyr Asn Gly Met Cys Gln Tyr Tyr Asp Ala Gln Cys Gln Val
             120                 125                 130

atc ttt ggc tca aaa gcc aag gct gcc ccc aaa gat tgt ttc att gaa   489
Ile Phe Gly Ser Lys Ala Lys Ala Ala Pro Lys Asp Cys Phe Ile Glu
         135                 140                 145

gtg aat tct aaa ggt gac aga ttt ggc aat tgt ggt ttc tct ggc aat   537
Val Asn Ser Lys Gly Asp Arg Phe Gly Asn Cys Gly Phe Ser Gly Asn
     150                 155                 160

gaa tac aag aag tgt gcc act ggg aat gct ttg tgt gga aag ctt cag   585
Glu Tyr Lys Lys Cys Ala Thr Gly Asn Ala Leu Cys Gly Lys Leu Gln
165                 170                 175                 180

tgt gag aat gta caa gag ata cct gta ttt gga att gtg cct gct att   633
Cys Glu Asn Val Gln Glu Ile Pro Val Phe Gly Ile Val Pro Ala Ile
                 185                 190                 195

att caa acg cct agt cga ggc acc aaa tgt tgg ggt gtg gat ttc cag   681
Ile Gln Thr Pro Ser Arg Gly Thr Lys Cys Trp Gly Val Asp Phe Gln
             200                 205                 210

cta gga tca gat gtt cca gat cct ggg atg gtt aac gaa ggc aca aaa   729
Leu Gly Ser Asp Val Pro Asp Pro Gly Met Val Asn Glu Gly Thr Lys
         215                 220                 225

tgt ggt gct gga aag atc tgt aga aac ttc cag tgt gta gat gct tct   777
Cys Gly Ala Gly Lys Ile Cys Arg Asn Phe Gln Cys Val Asp Ala Ser
     230                 235                 240

gtt ctg aat tat gac tgt gat gtt cag aaa aag tgt cat gga cat ggg   825
Val Leu Asn Tyr Asp Cys Asp Val Gln Lys Lys Cys His Gly His Gly
245                 250                 255                 260

gta tgt aat agc aat aag aat tgt cac tgt gaa aat ggc tgg gct ccc   873
Val Cys Asn Ser Asn Lys Asn Cys His Cys Glu Asn Gly Trp Ala Pro
                 265                 270                 275

cca aat tgt gag act aaa gga tac gga gga agt gtg gac agt gga cct   921
Pro Asn Cys Glu Thr Lys Gly Tyr Gly Gly Ser Val Asp Ser Gly Pro
             280                 285                 290
```

```
aca tac aat gaa atg aat act gca ttg agg gac gga tct tgt gac aaa   969

Thr Tyr Asn Glu Met Asn Thr Ala Leu Arg Asp Gly Ser Cys Asp Lys
        295                 300                 305

act cac aca tgc cca ccg tgc cca gca cct gaa gcc gag ggc gcg ccg   1017
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro
    310                 315                 320

tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc   1065
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
325                 330                 335                 340

cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac   1113
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
            345                 350                 355

cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat   1161
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
        360                 365                 370

gcc aag aca aag ccg cgg gag gag cag tac aac agc acg tac cgg gtg   1209
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
    375                 380                 385

gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag   1257
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
390                 395                 400

tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa   1305
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
405                 410                 415                 420

acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc   1353
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
            425                 430                 435

ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc   1401
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
        440                 445                 450

tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag   1449
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
    455                 460                 465

agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg   1497
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
470                 475                 480

gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag   1545
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
485                 490                 495                 500

agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag   1593
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
            505                 510                 515

gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt   1641
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
        520                 525                 530

aaa tga actagagcgg ccgctacaga t                                  1668
Lys
```

<210> 4

<211> 533
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: fusion polypeptide

<400> 4

```
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
  1               5                  10                  15
Gly Ser Thr Gly Thr Ser Cys Gly Asn Lys Leu Val Asp Ala Gly Glu
            20                  25                  30
Glu Cys Asp Cys Gly Thr Pro Lys Glu Cys Glu Leu Asp Pro Cys Cys
        35                  40                  45
Glu Gly Ser Thr Cys Lys Leu Lys Ser Phe Ala Glu Cys Ala Tyr Gly
    50                  55                  60
Asp Cys Cys Lys Asp Cys Arg Phe Leu Pro Gly Gly Thr Leu Cys Arg
65                  70                  75                  80
Gly Lys Thr Ser Glu Cys Asp Val Pro Glu Tyr Cys Asn Gly Ser Ser
                85                  90                  95
Gln Phe Cys Gln Pro Asp Val Phe Ile Gln Asn Gly Tyr Pro Cys Gln
            100                 105                 110
Asn Asn Lys Ala Tyr Cys Tyr Asn Gly Met Cys Gln Tyr Tyr Asp Ala
        115                 120                 125
Gln Cys Gln Val Ile Phe Gly Ser Lys Ala Lys Ala Ala Pro Lys Asp
    130                 135                 140
Cys Phe Ile Glu Val Asn Ser Lys Gly Asp Arg Phe Gly Asn Cys Gly
145                 150                 155                 160
Phe Ser Gly Asn Glu Tyr Lys Lys Cys Ala Thr Gly Asn Ala Leu Cys
                165                 170                 175
Gly Lys Leu Gln Cys Glu Asn Val Gln Glu Ile Pro Val Phe Gly Ile
            180                 185                 190
Val Pro Ala Ile Ile Gln Thr Pro Ser Arg Gly Thr Lys Cys Trp Gly
        195                 200                 205
Val Asp Phe Gln Leu Gly Ser Asp Val Pro Asp Pro Gly Met Val Asn
    210                 215                 220
Glu Gly Thr Lys Cys Gly Ala Gly Lys Ile Cys Arg Asn Phe Gln Cys
225                 230                 235                 240
Val Asp Ala Ser Val Leu Asn Tyr Asp Cys Asp Val Gln Lys Lys Cys
                245                 250                 255
His Gly His Gly Val Cys Asn Ser Asn Lys Asn Cys His Cys Glu Asn
            260                 265                 270
Gly Trp Ala Pro Pro Asn Cys Glu Thr Lys Gly Tyr Gly Gly Ser Val
        275                 280                 285
Asp Ser Gly Pro Thr Tyr Asn Glu Met Asn Thr Ala Leu Arg Asp Gly
    290                 295                 300
Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala
305                 310                 315                 320
Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
                325                 330                 335
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
            340                 345                 350
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
        355                 360                 365
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
    370                 375                 380
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
385                 390                 395                 400
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
                405                 410                 415
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
            420                 425                 430
Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
        435                 440                 445
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
    450                 455                 460
```

```
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
465                 470                 475                 480
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
                485                 490                 495
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
            500                 505                 510
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
        515                 520                 525
Leu Ser Pro Gly Lys
    530
```

<210> 5
<211> 1443
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: fusion polypeptide

<220>
<221> CDS
<222> (25)..(1422)

<400> 5

```
gtcgacccaa gctggctagc cacc atg gag aca gac aca ctc ctg cta tgg    51
                           Met Glu Thr Asp Thr Leu Leu Leu Trp
                             1               5

gta ctg ctg ctc tgg gtt cca ggt tcc act ggt act agt tgt gga aat   99
Val Leu Leu Leu Trp Val Pro Gly Ser Thr Gly Thr Ser Cys Gly Asn
 10                  15                  20                  25

gga atg gta gaa caa ggt gaa gaa tgt gat tgt ggc tat agt gac cag   147
Gly Met Val Glu Gln Gly Glu Glu Cys Asp Cys Gly Tyr Ser Asp Gln
                 30                  35                  40

tgt aaa gat gaa tgc tgc ttc gat gca aat caa cca gag gga aga aaa   195
Cys Lys Asp Glu Cys Cys Phe Asp Ala Asn Gln Pro Glu Gly Arg Lys
             45                  50                  55

tgc aaa ctg aaa cct ggg aaa cag tgc agt cca agt caa ggt cct tgt   243
Cys Lys Leu Lys Pro Gly Lys Gln Cys Ser Pro Ser Gln Gly Pro Cys
         60                  65                  70

tgt aca gca cag tgt gca ttc aag tca aag tct gag aag tgt cgg gat   291
Cys Thr Ala Gln Cys Ala Phe Lys Ser Lys Ser Glu Lys Cys Arg Asp
     75                  80                  85

gat tca gac tgt gca agg gaa gga ata tgt aat ggc ttc aca gct ctc   339
Asp Ser Asp Cys Ala Arg Glu Gly Ile Cys Asn Gly Phe Thr Ala Leu
 90                  95                  100                 105

tgc cca gca tct gac cct aaa cca aac ttc aca gac tgt aat agg cat   387
Cys Pro Ala Ser Asp Pro Lys Pro Asn Phe Thr Asp Cys Asn Arg His
                110                 115                 120

aca caa gtg tgc att aat ggg caa tgt gca ggt tct atc tgt gag aaa   435
Thr Gln Val Cys Ile Asn Gly Gln Cys Ala Gly Ser Ile Cys Glu Lys
            125                 130                 135

tat ggc tta gag gag tgt acg tgt gcc agt tct gat ggc aaa gat gat   483
Tyr Gly Leu Glu Glu Cys Thr Cys Ala Ser Ser Asp Gly Lys Asp Asp
        140                 145                 150
```

```
aaa gaa tta tgc cat gta tgc tgt atg aag aaa atg gac cca tca act   531
Lys Glu Leu Cys His Val Cys Cys Met Lys Lys Met Asp Pro Ser Thr
    155                 160                 165

tgt gcc agt aca ggg tct gtg cag tgg agt agg cac ttc agt ggt cga   579
Cys Ala Ser Thr Gly Ser Val Gln Trp Ser Arg His Phe Ser Gly Arg
170                 175                 180                 185

acc atc acc ctg caa cct gga tcc cct tgc aac gat ttt aga ggt tac   627
Thr Ile Thr Leu Gln Pro Gly Ser Pro Cys Asn Asp Phe Arg Gly Tyr
                190                 195                 200

tgt gat gtt ttc atg cgg tgc aga tta gta gat gct gat ggt cct cta   675
Cys Asp Val Phe Met Arg Cys Arg Leu Val Asp Ala Asp Gly Pro Leu
            205                 210                 215

gct agg ctt aaa aaa gca att ttt agt cca gag ctc tat gaa aac att   723
Ala Arg Leu Lys Lys Ala Ile Phe Ser Pro Glu Leu Tyr Glu Asn Ile
        220                 225                 230

gct gaa aga tct tgt gac aaa act cac aca tgc cca ccg tgc cca gca   771
Ala Glu Arg Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
    235                 240                 245

cct gaa gcc gag ggc gcg ccg tca gtc ttc ctc ttc ccc cca aaa ccc   819
Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
250                 255                 260                 265

aag gac acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg   867
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
                270                 275                 280

gtg gac gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg   915
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
            285                 290                 295

gac ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag   963
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
        300                 305                 310

tac aac agc acg tac cgg gtg gtc agc gtc ctc acc gtc ctg cac cag   1011
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
    315                 320                 325

gac tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc   1059
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
330                 335                 340                 345

ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc   1107
Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
                350                 355                 360

cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc   1155
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
            365                 370                 375

aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc   1203
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
        380                 385                 390

gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac   1251
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
    395                 400                 405

aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac   1299
```

```
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
410                 415                 420                 425

agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc   1347
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
                430                 435                 440

tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg cag aag   1395
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
            445                 450                 455

agc ctc tcc ctg tct ccg ggt aaa tga actagagcgg ccgctacaga t       1443
Ser Leu Ser Leu Ser Pro Gly Lys
        460                 465


<210> 6
<211> 465
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: fusion
      polypeptide

<400> 6
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
  1               5                  10                  15
Gly Ser Thr Gly Thr Ser Cys Gly Asn Gly Met Val Glu Gln Gly Glu
             20                  25                  30
Glu Cys Asp Cys Gly Tyr Ser Asp Gln Cys Lys Asp Glu Cys Cys Phe
         35                  40                  45
Asp Ala Asn Gln Pro Glu Gly Arg Lys Cys Lys Leu Lys Pro Gly Lys
     50                  55                  60
Gln Cys Ser Pro Ser Gln Gly Pro Cys Cys Thr Ala Gln Cys Ala Phe
 65                  70                  75                  80
Lys Ser Lys Ser Glu Lys Cys Arg Asp Asp Ser Asp Cys Ala Arg Glu
                 85                  90                  95
Gly Ile Cys Asn Gly Phe Thr Ala Leu Cys Pro Ala Ser Asp Pro Lys
            100                 105                 110
Pro Asn Phe Thr Asp Cys Asn Arg His Thr Gln Val Cys Ile Asn Gly
        115                 120                 125
Gln Cys Ala Gly Ser Ile Cys Glu Lys Tyr Gly Leu Glu Glu Cys Thr
    130                 135                 140
Cys Ala Ser Ser Asp Gly Lys Asp Asp Lys Glu Leu Cys His Val Cys
145                 150                 155                 160
Cys Met Lys Lys Met Asp Pro Ser Thr Cys Ala Ser Thr Gly Ser Val
                165                 170                 175
Gln Trp Ser Arg His Phe Ser Gly Arg Thr Ile Thr Leu Gln Pro Gly
            180                 185                 190
Ser Pro Cys Asn Asp Phe Arg Gly Tyr Cys Asp Val Phe Met Arg Cys
        195                 200                 205
Arg Leu Val Asp Ala Asp Gly Pro Leu Ala Arg Leu Lys Lys Ala Ile
    210                 215                 220
Phe Ser Pro Glu Leu Tyr Glu Asn Ile Ala Glu Arg Ser Cys Asp Lys
225                 230                 235                 240
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro
                245                 250                 255
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
            260                 265                 270
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
        275                 280                 285
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
    290                 295                 300
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
305                 310                 315                 320
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
                325                 330                 335
```

```
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
            340                 345                 350
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
        355                 360                 365
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
    370                 375                 380
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
385                 390                 395                 400
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
                405                 410                 415
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
            420                 425                 430
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
        435                 440                 445
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
    450                 455                 460
Lys
465
```

<210> 7
<211> 1638
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: fusion polypeptide

<220>
<221> CDS
<222> (41)..(1609)

<400> 7

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cgggcccccc ctcgaggtcg acccaagctg gctagccacc atg gag aca gac aca    55
                                                Met Glu Thr Asp Thr
                                                  1               5

ctc ctg cta tgg gta ctg ctg ctc tgg gtt cca ggt tcc act ggt act    103
Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro Gly Ser Thr Gly Thr
                10                  15                  20

agt tgc gga aat atg ttt gtg gag ccg ggc gag cag tgt gac tgt ggc    151
Ser Cys Gly Asn Met Phe Val Glu Pro Gly Glu Gln Cys Asp Cys Gly
            25                  30                  35

ttc ctg gat gac tgc gtc gat ccc tgc tgt gat tct ttg acc tgc cag    199
Phe Leu Asp Asp Cys Val Asp Pro Cys Cys Asp Ser Leu Thr Cys Gln
        40                  45                  50

ctg agg cca ggt gca cag tgt gca tct gac gga ccc tgt tgt caa aat    247
Leu Arg Pro Gly Ala Gln Cys Ala Ser Asp Gly Pro Cys Cys Gln Asn
    55                  60                  65

tgc cag ctg cgc ccg tct ggc tgg cag tgt cgt cct acc aga ggg gat    295
Cys Gln Leu Arg Pro Ser Gly Trp Gln Cys Arg Pro Thr Arg Gly Asp
 70                  75                  80                  85

tgt gac ttg cct gaa ttc tgc cca gga gac agc tcc cag tgt ccc cct    343
Cys Asp Leu Pro Glu Phe Cys Pro Gly Asp Ser Ser Gln Cys Pro Pro
                90                  95                  100

gat gtc agc cta ggg gat ggc gag ccc tgc gct ggc ggg caa gct gtg    391
Asp Val Ser Leu Gly Asp Gly Glu Pro Cys Ala Gly Gly Gln Ala Val
            105                 110                 115
```

```
tgc atg cac ggg cgt tgt gcc tcc tat gcc cag cag tgc cag tca ctt   439

Cys Met His Gly Arg Cys Ala Ser Tyr Ala Gln Gln Cys Gln Ser Leu
        120                 125                 130

tgg gga cct gga gcc cag ccc gct gcg cca ctt tgc ctc cag aca gct   487
Trp Gly Pro Gly Ala Gln Pro Ala Ala Pro Leu Cys Leu Gln Thr Ala
    135                 140                 145

aat act cgg gga aat gct ttt ggg agc tgt ggg cgc aac ccc agt ggc   535
Asn Thr Arg Gly Asn Ala Phe Gly Ser Cys Gly Arg Asn Pro Ser Gly
150                 155                 160                 165

agt tat gtg tcc tgc acc cct aga gat gcc att tgt ggg cag ctc cag   583
Ser Tyr Val Ser Cys Thr Pro Arg Asp Ala Ile Cys Gly Gln Leu Gln
            170                 175                 180

tgc cag aca ggt agg acc cag cct ctg ctg ggc tcc atc cgg gat cta   631
Cys Gln Thr Gly Arg Thr Gln Pro Leu Leu Gly Ser Ile Arg Asp Leu
        185                 190                 195

ctc tgg gag aca ata gat gtg aat ggg act gag ctg aac tgc agc tgg   679
Leu Trp Glu Thr Ile Asp Val Asn Gly Thr Glu Leu Asn Cys Ser Trp
    200                 205                 210

gtg cac ctg gac ctg ggc agt gat gtg gcc cag ccc ctc ctg act ctg   727
Val His Leu Asp Leu Gly Ser Asp Val Ala Gln Pro Leu Leu Thr Leu
215                 220                 225

cct ggc aca gcc tgt ggc cct ggc ctg gtg tgt ata gac cat cga tgc   775
Pro Gly Thr Ala Cys Gly Pro Gly Leu Val Cys Ile Asp His Arg Cys
230                 235                 240                 245

cag cgt gtg gat ctc ctg ggg gca cag gaa tgt cga agc aaa tgc cat   823
Gln Arg Val Asp Leu Leu Gly Ala Gln Glu Cys Arg Ser Lys Cys His
            250                 255                 260

gga cat ggg gtc tgt gac agc aac agg cac tgc tac tgt gag gag ggc   871
Gly His Gly Val Cys Asp Ser Asn Arg His Cys Tyr Cys Glu Glu Gly
        265                 270                 275

tgg gca ccc cct gac tgc acc act cag ctc aaa gca acc agc tcc aga   919
Trp Ala Pro Pro Asp Cys Thr Thr Gln Leu Lys Ala Thr Ser Ser Arg
    280                 285                 290

tct tgt gac aaa act cac aca tgc cca ccg tgc cca gca cct gaa gcc   967
Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala
295                 300                 305

gag ggc gcg ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc   1015
Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
310                 315                 320                 325

ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg   1063
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
            330                 335                 340

agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg   1111
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
        345                 350                 355

gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac agc   1159
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
    360                 365                 370

acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg   1207
```

```
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
    375                 380                 385

aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc   1255
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
390                 395                 400                 405

ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca   1303
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
                410                 415                 420

cag gtg tac acc ctg ccc cca tcc cgg gag gag atg acc aag aac cag   1351
Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
            425                 430                 435

gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc   1399
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
        440                 445                 450

gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg   1447
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
    455                 460                 465

cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tat agc aag ctc   1495
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
470                 475                 480                 485

acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc   1543
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
                490                 495                 500

gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc   1591
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
            505                 510                 515

ctg tct ccg ggt aaa tga actagagcgg ccgccaccgc ggtggagct          1638
Leu Ser Pro Gly Lys
        520
```

<210> 8
<211> 522
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: fusion polypeptide

<400> 8

```
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
  1               5                  10                  15
Gly Ser Thr Gly Thr Ser Cys Gly Asn Met Phe Val Glu Pro Gly Glu
             20                  25                  30
Gln Cys Asp Cys Gly Phe Leu Asp Asp Cys Val Asp Pro Cys Cys Asp
         35                  40                  45
Ser Leu Thr Cys Gln Leu Arg Pro Gly Ala Gln Cys Ala Ser Asp Gly
     50                  55                  60
Pro Cys Cys Gln Asn Cys Gln Leu Arg Pro Ser Gly Trp Gln Cys Arg
 65                  70                  75                  80
Pro Thr Arg Gly Asp Cys Asp Leu Pro Glu Phe Cys Pro Gly Asp Ser
                 85                  90                  95
Ser Gln Cys Pro Pro Asp Val Ser Leu Gly Asp Gly Glu Pro Cys Ala
            100                 105                 110
Gly Gly Gln Ala Val Cys Met His Gly Arg Cys Ala Ser Tyr Ala Gln
        115                 120                 125
Gln Cys Gln Ser Leu Trp Gly Pro Gly Ala Gln Pro Ala Ala Pro Leu
    130                 135                 140
```

```
Cys Leu Gln Thr Ala Asn Thr Arg Gly Asn Ala Phe Gly Ser Cys Gly
145                 150                 155                 160
Arg Asn Pro Ser Gly Ser Tyr Val Ser Cys Thr Pro Arg Asp Ala Ile
                165                 170                 175
Cys Gly Gln Leu Gln Cys Gln Thr Gly Arg Thr Gln Pro Leu Leu Gly
            180                 185                 190
Ser Ile Arg Asp Leu Leu Trp Glu Thr Ile Asp Val Asn Gly Thr Glu
        195                 200                 205
Leu Asn Cys Ser Trp Val His Leu Asp Leu Gly Ser Asp Val Ala Gln
    210                 215                 220
Pro Leu Leu Thr Leu Pro Gly Thr Ala Cys Gly Pro Gly Leu Val Cys
225                 230                 235                 240
Ile Asp His Arg Cys Gln Arg Val Asp Leu Leu Gly Ala Gln Glu Cys
                245                 250                 255
Arg Ser Lys Cys His Gly His Gly Val Cys Asp Ser Asn Arg His Cys
            260                 265                 270
Tyr Cys Glu Glu Gly Trp Ala Pro Pro Asp Cys Thr Thr Gln Leu Lys
        275                 280                 285
Ala Thr Ser Ser Arg Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
    290                 295                 300
Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro
305                 310                 315                 320
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
                325                 330                 335
Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
            340                 345                 350
Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
        355                 360                 365
Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
    370                 375                 380
His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
385                 390                 395                 400
Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
                405                 410                 415
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
            420                 425                 430
Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
        435                 440                 445
Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
    450                 455                 460
Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
465                 470                 475                 480
Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
                485                 490                 495
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
            500                 505                 510
Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        515                 520
```

<210> 9
<211> 1386
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: fusion polypeptide

<220>
<221> CDS
<222> (25)..(1365)

<400> 9
gtcgacccaa gctggctagc cacc atg gag aca gac aca ctc ctg cta tgg 51

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                                Met Glu Thr Asp Thr Leu Leu Leu Trp
                                  1               5

gta ctg ctg ctc tgg gtt cca ggt tcc act ggt act agt tgt ggg aac   99
Val Leu Leu Leu Trp Val Pro Gly Ser Thr Gly Thr Ser Cys Gly Asn
 10                  15                  20                  25

tcg agg gtg gat gaa gga gaa gag tgt gat cct ggc atc atg tat ctg   147
Ser Arg Val Asp Glu Gly Glu Glu Cys Asp Pro Gly Ile Met Tyr Leu
                 30                  35                  40

aac aac gac acc tgc tgc aac agc gac tgc acg ttg aag gaa ggt gtc   195
Asn Asn Asp Thr Cys Cys Asn Ser Asp Cys Thr Leu Lys Glu Gly Val
             45                  50                  55

cag tgc agt gac agg aac agt cct tgc tgt aaa aac tgt cag ttt gag   243
Gln Cys Ser Asp Arg Asn Ser Pro Cys Cys Lys Asn Cys Gln Phe Glu
         60                  65                  70

act gcc cag aag aag tgc cag gag gcg att aat gct act tgc aaa ggc   291
Thr Ala Gln Lys Lys Cys Gln Glu Ala Ile Asn Ala Thr Cys Lys Gly
     75                  80                  85

gtg tcc tac tgc aca ggt aat agc agt gag tgc ccg cct cca gga aat   339
Val Ser Tyr Cys Thr Gly Asn Ser Ser Glu Cys Pro Pro Pro Gly Asn
 90                  95                  100                 105

gct gaa gat gac act gtt tgc ttg gat ctt ggc aag tgt aag gat ggg   387
Ala Glu Asp Asp Thr Val Cys Leu Asp Leu Gly Lys Cys Lys Asp Gly
                110                 115                 120

aaa tgc atc cct ttc tgc gag agg gaa cag cag ctg gag tcc tgt gca   435
Lys Cys Ile Pro Phe Cys Glu Arg Glu Gln Gln Leu Glu Ser Cys Ala
            125                 130                 135

tgt aat gaa act gac aac tcc tgc aag gtg tgc tgc agg gac ctt tcc   483
Cys Asn Glu Thr Asp Asn Ser Cys Lys Val Cys Cys Arg Asp Leu Ser
        140                 145                 150

ggc cgc tgt gtg ccc tat gtc gat gct gaa caa aag aac tta ttt ttg   531
Gly Arg Cys Val Pro Tyr Val Asp Ala Glu Gln Lys Asn Leu Phe Leu
    155                 160                 165

agg aaa gga aag ccc tgt aca gta gga ttt tgt gac atg aat ggc aaa   579
Arg Lys Gly Lys Pro Cys Thr Val Gly Phe Cys Asp Met Asn Gly Lys
170                 175                 180                 185

tgt gag aaa cga gta cag gat gta att gaa cga ttt tgg gat ttc att   627
Cys Glu Lys Arg Val Gln Asp Val Ile Glu Arg Phe Trp Asp Phe Ile
                190                 195                 200

gac cag ctg agc atc aat act ttt gga aag ttt tta gca gac aac aga   675
Asp Gln Leu Ser Ile Asn Thr Phe Gly Lys Phe Leu Ala Asp Asn Arg
            205                 210                 215

tct tgt gac aaa act cac aca tgc cca ccg tgc cca gca cct gaa gcc   723
Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala
        220                 225                 230

gag ggc gcg ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc   771
Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
    235                 240                 245

ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg   819
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
250                 255                 260                 265
```

```
agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg   867
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
                270                 275                 280

gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac agc   915
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
            285                 290                 295

acg tac cgg gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg   963
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
        300                 305                 310

aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc   1011
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
    315                 320                 325

ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca   1059
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
330                 335                 340                 345

cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag   1107
Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
                350                 355                 360

gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc   1155
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
            365                 370                 375

gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg   1203
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
        380                 385                 390

cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc   1251
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
    395                 400                 405

acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc   1299
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
410                 415                 420                 425

gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc   1347
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
                430                 435                 440

ctg tct ccg ggt aaa tga actagagcgg ccgctacaga t                  1386
Leu Ser Pro Gly Lys
            445
```

<210> 10
<211> 446
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: fusion polypeptide

<400> 10

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Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
  1               5                  10                  15
Gly Ser Thr Gly Thr Ser Cys Gly Asn Ser Arg Val Asp Glu Gly Glu
            20                  25                  30
Glu Cys Asp Pro Gly Ile Met Tyr Leu Asn Asn Asp Thr Cys Cys Asn
        35                  40                  45
Ser Asp Cys Thr Leu Lys Glu Gly Val Gln Cys Ser Asp Arg Asn Ser
    50                  55                  60
```

```
Pro Cys Cys Lys Asn Cys Gln Phe Glu Thr Ala Gln Lys Lys Cys Gln
 65                  70                  75                  80
Glu Ala Ile Asn Ala Thr Cys Lys Gly Val Ser Tyr Cys Thr Gly Asn
                 85                  90                  95
Ser Ser Glu Cys Pro Pro Pro Gly Asn Ala Glu Asp Asp Thr Val Cys
            100                 105                 110
Leu Asp Leu Gly Lys Cys Lys Asp Gly Lys Cys Ile Pro Phe Cys Glu
        115                 120                 125
Arg Glu Gln Gln Leu Glu Ser Cys Ala Cys Asn Glu Thr Asp Asn Ser
    130                 135                 140
Cys Lys Val Cys Cys Arg Asp Leu Ser Gly Arg Cys Val Pro Tyr Val
145                 150                 155                 160
Asp Ala Glu Gln Lys Asn Leu Phe Leu Arg Lys Gly Lys Pro Cys Thr
                165                 170                 175
Val Gly Phe Cys Asp Met Asn Gly Lys Cys Glu Lys Arg Val Gln Asp
            180                 185                 190
Val Ile Glu Arg Phe Trp Asp Phe Ile Asp Gln Leu Ser Ile Asn Thr
        195                 200                 205
Phe Gly Lys Phe Leu Ala Asp Asn Arg Ser Cys Asp Lys Thr His Thr
    210                 215                 220
Cys Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe
225                 230                 235                 240
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
                245                 250                 255
Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
            260                 265                 270
Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
        275                 280                 285
Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
    290                 295                 300
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
305                 310                 315                 320
Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
                325                 330                 335
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
            340                 345                 350
Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
        355                 360                 365
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
    370                 375                 380
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
385                 390                 395                 400
Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
                405                 410                 415
Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
            420                 425                 430
Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440                 445
```

<210> 11
<211> 1653
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: fusion polypeptide

<220>
<221> CDS
<222> (25)..(1632)

<400> 11
gtcgacccaa gctggctagc cacc atg gag aca gac aca ctc ctg cta tgg 51

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                                Met Glu Thr Asp Thr Leu Leu Leu Trp
                                  1               5

gta ctg ctg ctc tgg gtt cca ggt tcc act ggt act agt tgt ggg aat   99
Val Leu Leu Leu Trp Val Pro Gly Ser Thr Gly Thr Ser Cys Gly Asn
 10                  15                  20                  25

cta gtg gtt gaa gaa ggg gag gaa tgt gac tgt gga acc ata cgg cag   147
Leu Val Val Glu Glu Gly Glu Glu Cys Asp Cys Gly Thr Ile Arg Gln
                 30                  35                  40

tgt gca aaa gat ccc tgt tgt ctg tta aac tgt act cta cat cct ggg   195
Cys Ala Lys Asp Pro Cys Cys Leu Leu Asn Cys Thr Leu His Pro Gly
             45                  50                  55

gct gct tgt gct ttt gga ata tgt tgc aaa gac tgc aaa ttt ctg cca   243
Ala Ala Cys Ala Phe Gly Ile Cys Cys Lys Asp Cys Lys Phe Leu Pro
         60                  65                  70

tca gga act tta tgt aga caa caa gtt ggt gaa tgt gac ctt cca gag   291
Ser Gly Thr Leu Cys Arg Gln Gln Val Gly Glu Cys Asp Leu Pro Glu
     75                  80                  85

tgg tgc aat ggg aca tcc cat caa tgc cca gat gat gtg tat gtg cag   339
Trp Cys Asn Gly Thr Ser His Gln Cys Pro Asp Asp Val Tyr Val Gln
 90                  95                  100                 105

gac ggg atc tcc tgt aat gtg aat gcc ttc tgc tat gaa aag acg tgt   387
Asp Gly Ile Ser Cys Asn Val Asn Ala Phe Cys Tyr Glu Lys Thr Cys
                110                 115                 120

aat aac cat gat ata caa tgt aaa gag att ttt ggc caa gat gca agg   435
Asn Asn His Asp Ile Gln Cys Lys Glu Ile Phe Gly Gln Asp Ala Arg
            125                 130                 135

agt gca tct cag agt tgc tac caa gaa atc aac acc caa gga aac cgt   483
Ser Ala Ser Gln Ser Cys Tyr Gln Glu Ile Asn Thr Gln Gly Asn Arg
        140                 145                 150

ttc ggt cac tgt ggt att gta ggc aca aca tat gta aaa tgt tgg acc   531
Phe Gly His Cys Gly Ile Val Gly Thr Thr Tyr Val Lys Cys Trp Thr
    155                 160                 165

cct gat atc atg tgt ggg agg gtt cag tgt gaa aat gtg gga gta att   579
Pro Asp Ile Met Cys Gly Arg Val Gln Cys Glu Asn Val Gly Val Ile
170                 175                 180                 185

ccc aat ctg ata gag cat tct aca gtg cag cag ttt cac ctc aat gac   627
Pro Asn Leu Ile Glu His Ser Thr Val Gln Gln Phe His Leu Asn Asp
                190                 195                 200

acc act tgc tgg ggc act gat tat cat tta ggg atg gct ata cct gat   675
Thr Thr Cys Trp Gly Thr Asp Tyr His Leu Gly Met Ala Ile Pro Asp
            205                 210                 215

att ggt gag gtg aaa gat ggc aca gta tgt ggt cca gaa aag atc tgc   723
Ile Gly Glu Val Lys Asp Gly Thr Val Cys Gly Pro Glu Lys Ile Cys
        220                 225                 230

atc cgt aag aag tgt gcc agt atg gtt cat ctg tca caa gcc tgt cag   771
Ile Arg Lys Lys Cys Ala Ser Met Val His Leu Ser Gln Ala Cys Gln
    235                 240                 245

cct aag acc tgc aac atg agg gga atc tgc aac aac aaa caa cac tgt   819
Pro Lys Thr Cys Asn Met Arg Gly Ile Cys Asn Asn Lys Gln His Cys
250                 255                 260                 265
```

```
cac tgc aac cat gaa tgg gca ccc cca tac tgc aag gac aaa ggc tat   867
His Cys Asn His Glu Trp Ala Pro Pro Tyr Cys Lys Asp Lys Gly Tyr
                270                 275                 280

gga ggt agt gct gat agt ggc cca cct cct aag aac aac atg gaa gga   915
Gly Gly Ser Ala Asp Ser Gly Pro Pro Pro Lys Asn Asn Met Glu Gly
            285                 290                 295

tta aat gtg atg gga aag ttg cgt gga tct tgt gac aaa act cac aca   963
Leu Asn Val Met Gly Lys Leu Arg Gly Ser Cys Asp Lys Thr His Thr
        300                 305                 310

tgc cca ccg tgc cca gca cct gaa gcc gag ggc gcg ccg tca gtc ttc   1011
Cys Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe
    315                 320                 325

ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg acc cct   1059
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
330                 335                 340                 345

gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc   1107
Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
                350                 355                 360

aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag aca   1155
Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
            365                 370                 375

aag ccg cgg gag gag cag tac aac agc acg tac cgg gtg gtc agc gtc   1203
Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
        380                 385                 390

ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc   1251
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
    395                 400                 405

aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc   1299
Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
410                 415                 420                 425

aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca   1347
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
                430                 435                 440

tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc   1395
Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
            445                 450                 455

aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg   1443
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
        460                 465                 470

cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac   1491
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
    475                 480                 485

ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag agc agg tgg   1539
Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
490                 495                 500                 505

cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac   1587
Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
                510                 515                 520

aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa tga       1632
```

```
Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
            525                 530                 535

actagagcgg ccgctacaga t                                          1653


<210> 12
<211> 535
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: fusion
      polypeptide

<400> 12
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
  1               5                  10                  15
Gly Ser Thr Gly Thr Ser Cys Gly Asn Leu Val Val Glu Glu Gly Glu
             20                  25                  30
Glu Cys Asp Cys Gly Thr Ile Arg Gln Cys Ala Lys Asp Pro Cys Cys
         35                  40                  45
Leu Leu Asn Cys Thr Leu His Pro Gly Ala Ala Cys Ala Phe Gly Ile
     50                  55                  60
Cys Cys Lys Asp Cys Lys Phe Leu Pro Ser Gly Thr Leu Cys Arg Gln
 65                  70                  75                  80
Gln Val Gly Glu Cys Asp Leu Pro Glu Trp Cys Asn Gly Thr Ser His
                 85                  90                  95
Gln Cys Pro Asp Asp Val Tyr Val Gln Asp Gly Ile Ser Cys Asn Val
            100                 105                 110
Asn Ala Phe Cys Tyr Glu Lys Thr Cys Asn Asn His Asp Ile Gln Cys
        115                 120                 125
Lys Glu Ile Phe Gly Gln Asp Ala Arg Ser Ala Ser Gln Ser Cys Tyr
    130                 135                 140
Gln Glu Ile Asn Thr Gln Gly Asn Arg Phe Gly His Cys Gly Ile Val
145                 150                 155                 160
Gly Thr Thr Tyr Val Lys Cys Trp Thr Pro Asp Ile Met Cys Gly Arg
                165                 170                 175
Val Gln Cys Glu Asn Val Gly Val Ile Pro Asn Leu Ile Glu His Ser
            180                 185                 190
Thr Val Gln Gln Phe His Leu Asn Asp Thr Thr Cys Trp Gly Thr Asp
        195                 200                 205
Tyr His Leu Gly Met Ala Ile Pro Asp Ile Gly Glu Val Lys Asp Gly
    210                 215                 220
Thr Val Cys Gly Pro Glu Lys Ile Cys Ile Arg Lys Lys Cys Ala Ser
225                 230                 235                 240
Met Val His Leu Ser Gln Ala Cys Gln Pro Lys Thr Cys Asn Met Arg
                245                 250                 255
Gly Ile Cys Asn Asn Lys Gln His Cys His Cys Asn His Glu Trp Ala
            260                 265                 270
Pro Pro Tyr Cys Lys Asp Lys Gly Tyr Gly Gly Ser Ala Asp Ser Gly
        275                 280                 285
Pro Pro Pro Lys Asn Asn Met Glu Gly Leu Asn Val Met Gly Lys Leu
    290                 295                 300
Arg Gly Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
305                 310                 315                 320
Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
                325                 330                 335
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
            340                 345                 350
Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
        355                 360                 365
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
    370                 375                 380
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
385                 390                 395                 400
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
                405                 410                 415
```

```
Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
            420                 425                 430
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
        435                 440                 445
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
    450                 455                 460
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
465                 470                 475                 480
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
                485                 490                 495
Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
            500                 505                 510
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
        515                 520                 525
Leu Ser Leu Ser Pro Gly Lys
    530                 535
```

<210> 13
<211> 1617
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: fusion polypeptide

<220>
<221> CDS
<222> (25)..(1596)

<400> 13

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gtcgacccaa gctggctagc cacc atg gag aca gac aca ctc ctg cta tgg    51
                           Met Glu Thr Asp Thr Leu Leu Leu Trp
                             1               5

gta ctg ctg ctc tgg gtt cca ggt tcc act ggt act agt tgt ggg aat   99
Val Leu Leu Leu Trp Val Pro Gly Ser Thr Gly Thr Ser Cys Gly Asn
 10                  15                  20                  25

ggt gtg gtt gaa aga gaa gag cag tgt gac tgt gga tcc gta cag cag   147
Gly Val Val Glu Arg Glu Glu Gln Cys Asp Cys Gly Ser Val Gln Gln
                 30                  35                  40

tgt gaa caa gac gcc tgt tgt ctg ttg aac tgc act cta agg cct ggg   195
Cys Glu Gln Asp Ala Cys Cys Leu Leu Asn Cys Thr Leu Arg Pro Gly
             45                  50                  55

gct gcc tgt gct ttt ggg ctt tgt tgc aaa gac tgc aag ttc atg cca   243
Ala Ala Cys Ala Phe Gly Leu Cys Cys Lys Asp Cys Lys Phe Met Pro
         60                  65                  70

tca ggg gaa ctc tgt aga caa gag gtc aat gaa tgt gac ctt cca gaa   291
Ser Gly Glu Leu Cys Arg Gln Glu Val Asn Glu Cys Asp Leu Pro Glu
     75                  80                  85

tgg tgc aat gga aca tct cat cag tgt cca gaa gat aga tat gtg cag   339
Trp Cys Asn Gly Thr Ser His Gln Cys Pro Glu Asp Arg Tyr Val Gln
 90                  95                  100                 105

gac ggg atc ccc tgt agt gac agt gcc tac tgc tat caa aag agg tgt   387
Asp Gly Ile Pro Cys Ser Asp Ser Ala Tyr Cys Tyr Gln Lys Arg Cys
                110                 115                 120

aat aac cat gac cag cat tgc agg gag att ttt ggt aaa gat gca aaa   435
```

```
Asn Asn His Asp Gln His Cys Arg Glu Ile Phe Gly Lys Asp Ala Lys
            125                 130                 135

agt gca tct cag aat tgc tat aaa gaa atc aac tct cag gga aac cgt   483
Ser Ala Ser Gln Asn Cys Tyr Lys Glu Ile Asn Ser Gln Gly Asn Arg
        140                 145                 150

ttt ggt cac tgt ggt ata aat ggc aca aca tac cta aaa tgt cat atc   531
Phe Gly His Cys Gly Ile Asn Gly Thr Thr Tyr Leu Lys Cys His Ile
    155                 160                 165

tct gat gtc ttt tgt ggg aga gtt caa tgt gag aat gtg aga gac att   579
Ser Asp Val Phe Cys Gly Arg Val Gln Cys Glu Asn Val Arg Asp Ile
170                 175                 180                 185

cct ctt ctc caa gat cat ttt act ttg cag cac act cat atc aat ggt   627
Pro Leu Leu Gln Asp His Phe Thr Leu Gln His Thr His Ile Asn Gly
                190                 195                 200

gtc acc tgc tgg ggt att gac tat cat tta agg atg aac ata tct gac   675
Val Thr Cys Trp Gly Ile Asp Tyr His Leu Arg Met Asn Ile Ser Asp
            205                 210                 215

att ggt gaa gtg aaa gat ggt act gtg tgt ggc cca gga aag atc tgc   723
Ile Gly Glu Val Lys Asp Gly Thr Val Cys Gly Pro Gly Lys Ile Cys
        220                 225                 230

atc cat aag aag tgt gtc agt ctg tct gtc ttg tca cat gtc tgc ctt   771
Ile His Lys Lys Cys Val Ser Leu Ser Val Leu Ser His Val Cys Leu
    235                 240                 245

cct gag acc tgc aat atg aag ggg atc tgc aat aac aaa cat cac tgc   819
Pro Glu Thr Cys Asn Met Lys Gly Ile Cys Asn Asn Lys His His Cys
250                 255                 260                 265

cac tgt ggc tat ggg tgg tcc cca ccc tac tgc cag cac aga ggc tat   867
His Cys Gly Tyr Gly Trp Ser Pro Pro Tyr Cys Gln His Arg Gly Tyr
                270                 275                 280

ggg ggc agt att gac agt ggc cca gca tct gca aag aga tct tgt gac   915
Gly Gly Ser Ile Asp Ser Gly Pro Ala Ser Ala Lys Arg Ser Cys Asp
            285                 290                 295

aaa act cac aca tgc cca ccg tgc cca gca cct gaa gcc gag ggc gcg   963
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala
        300                 305                 310

ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc   1011
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
    315                 320                 325

tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa   1059
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
330                 335                 340                 345

gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat   1107
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
                350                 355                 360

aat gcc aag aca aag ccg cgg gag gag cag tac aac agc acg tac cgg   1155
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
            365                 370                 375

gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag   1203
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
        380                 385                 390
```

```
gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag   1251
Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
    395                 400                 405

aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac   1299
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
410                 415                 420                 425

acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg   1347
Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
                430                 435                 440

acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg   1395
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
            445                 450                 455

gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg   1443
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
        460                 465                 470

ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac   1491
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
    475                 480                 485

aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat   1539
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
490                 495                 500                 505

gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg   1587
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
                510                 515                 520

ggt aaa tga actagagcgg ccgctacaga t                              1617
Gly Lys
```

<210> 14
<211> 523
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: fusion polypeptide

<400> 14

```
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
  1               5                  10                  15
Gly Ser Thr Gly Thr Ser Cys Gly Asn Gly Val Val Glu Arg Glu Glu
             20                  25                  30
Gln Cys Asp Cys Gly Ser Val Gln Gln Cys Glu Gln Asp Ala Cys Cys
         35                  40                  45
Leu Leu Asn Cys Thr Leu Arg Pro Gly Ala Ala Cys Ala Phe Gly Leu
     50                  55                  60
Cys Cys Lys Asp Cys Lys Phe Met Pro Ser Gly Glu Leu Cys Arg Gln
 65                  70                  75                  80
Glu Val Asn Glu Cys Asp Leu Pro Glu Trp Cys Asn Gly Thr Ser His
                 85                  90                  95
Gln Cys Pro Glu Asp Arg Tyr Val Gln Asp Gly Ile Pro Cys Ser Asp
            100                 105                 110
Ser Ala Tyr Cys Tyr Gln Lys Arg Cys Asn Asn His Asp Gln His Cys
        115                 120                 125
Arg Glu Ile Phe Gly Lys Asp Ala Lys Ser Ala Ser Gln Asn Cys Tyr
    130                 135                 140
Lys Glu Ile Asn Ser Gln Gly Asn Arg Phe Gly His Cys Gly Ile Asn
145                 150                 155                 160
Gly Thr Thr Tyr Leu Lys Cys His Ile Ser Asp Val Phe Cys Gly Arg
                165                 170                 175
```

```
Val Gln Cys Glu Asn Val Arg Asp Ile Pro Leu Leu Gln Asp His Phe
            180                 185                 190
Thr Leu Gln His Thr His Ile Asn Gly Val Thr Cys Trp Gly Ile Asp
        195                 200                 205
Tyr His Leu Arg Met Asn Ile Ser Asp Ile Gly Glu Val Lys Asp Gly
    210                 215                 220
Thr Val Cys Gly Pro Gly Lys Ile Cys Ile His Lys Lys Cys Val Ser
225                 230                 235                 240
Leu Ser Val Leu Ser His Val Cys Leu Pro Glu Thr Cys Asn Met Lys
                245                 250                 255
Gly Ile Cys Asn Asn Lys His His Cys His Cys Gly Tyr Gly Trp Ser
            260                 265                 270
Pro Pro Tyr Cys Gln His Arg Gly Tyr Gly Gly Ser Ile Asp Ser Gly
        275                 280                 285
Pro Ala Ser Ala Lys Arg Ser Cys Asp Lys Thr His Thr Cys Pro Pro
    290                 295                 300
Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro
305                 310                 315                 320
Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
                325                 330                 335
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
            340                 345                 350
Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
        355                 360                 365
Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
    370                 375                 380
Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
385                 390                 395                 400
Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
                405                 410                 415
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
            420                 425                 430
Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
        435                 440                 445
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
    450                 455                 460
Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
465                 470                 475                 480
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
                485                 490                 495
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
            500                 505                 510
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        515                 520
```

<210> 15
<211> 1674
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: fusion polypeptide

<220>
<221> CDS
<222> (25)..(1653)

<400> 15

```
gtcgacccaa gctggctagc cacc atg gag aca gac aca ctc ctg cta tgg    51
                           Met Glu Thr Asp Thr Leu Leu Leu Trp
                             1               5

gta ctg ctg ctc tgg gtt cca ggt tcc act ggt act agt tgt ggc aat   99
```

```
Val Leu Leu Leu Trp Val Pro Gly Ser Thr Gly Thr Ser Cys Gly Asn
 10                  15                  20                  25

ggc ttc att gaa act gga gag gag tgt gat tgt gga acc ccg gcc gaa   147
Gly Phe Ile Glu Thr Gly Glu Glu Cys Asp Cys Gly Thr Pro Ala Glu
                 30                  35                  40

tgt gtc ctt gaa gga gca gag tgt tgt aag aaa tgc acc ttg act caa   195
Cys Val Leu Glu Gly Ala Glu Cys Cys Lys Lys Cys Thr Leu Thr Gln
             45                  50                  55

gac tct caa tgc agt gac ggt ctt tgc tgt aaa aag tgc aag ttt cag   243
Asp Ser Gln Cys Ser Asp Gly Leu Cys Cys Lys Lys Cys Lys Phe Gln
         60                  65                  70

cct atg ggc act gtg tgc cga gaa gca gta aat gat tgt gat att cgt   291
Pro Met Gly Thr Val Cys Arg Glu Ala Val Asn Asp Cys Asp Ile Arg
     75                  80                  85

gaa acg tgc tca gga aat tca agc cag tgt gcc cct aat att cat aaa   339
Glu Thr Cys Ser Gly Asn Ser Ser Gln Cys Ala Pro Asn Ile His Lys
 90                  95                  100                 105

atg gat gga tat tca tgt gat ggt gtt cag gga att tgc ttt gga gga   387
Met Asp Gly Tyr Ser Cys Asp Gly Val Gln Gly Ile Cys Phe Gly Gly
                110                 115                 120

aga tgc aaa acc aga gat aga caa tgc aaa tac att tgg ggg caa aag   435
Arg Cys Lys Thr Arg Asp Arg Gln Cys Lys Tyr Ile Trp Gly Gln Lys
            125                 130                 135

gtg aca gca tca gac aaa tat tgc tat gag aaa ctg aat att gaa ggg   483
Val Thr Ala Ser Asp Lys Tyr Cys Tyr Glu Lys Leu Asn Ile Glu Gly
        140                 145                 150

acg gag aag ggt aac tgt ggg aaa gac aaa gac aca tgg ata cag tgc   531
Thr Glu Lys Gly Asn Cys Gly Lys Asp Lys Asp Thr Trp Ile Gln Cys
    155                 160                 165

aac aaa cgg gat gtg ctt tgt ggt tac ctt ttg tgt acc aat att ggc   579
Asn Lys Arg Asp Val Leu Cys Gly Tyr Leu Leu Cys Thr Asn Ile Gly
170                 175                 180                 185

aat atc cca agg ctt gga gaa ctc gat ggt gaa atc aca tct act tta   627
Asn Ile Pro Arg Leu Gly Glu Leu Asp Gly Glu Ile Thr Ser Thr Leu
                190                 195                 200

gtt gtg cag caa gga aga aca tta aac tgc agt ggt ggg cat gtt aag   675
Val Val Gln Gln Gly Arg Thr Leu Asn Cys Ser Gly Gly His Val Lys
            205                 210                 215

ctt gaa gaa gat gta gat ctt ggc tat gtg gaa gat ggg aca cct tgt   723
Leu Glu Glu Asp Val Asp Leu Gly Tyr Val Glu Asp Gly Thr Pro Cys
        220                 225                 230

ggt ccc caa atg atg tgc tta gaa cac agg tgt ctt cct gtg gct tct   771
Gly Pro Gln Met Met Cys Leu Glu His Arg Cys Leu Pro Val Ala Ser
    235                 240                 245

ttc aac ttt agt act tgc ttg agc agt aaa gaa ggc act att tgc tca   819
Phe Asn Phe Ser Thr Cys Leu Ser Ser Lys Glu Gly Thr Ile Cys Ser
250                 255                 260                 265

gga aat gga gtt tgc agt aat gag ctg aag tgt gtg tgt aac aga cac   867
Gly Asn Gly Val Cys Ser Asn Glu Leu Lys Cys Val Cys Asn Arg His
                270                 275                 280
```

```
tgg ata ggt tct gat tgc aac act tac ttc cct cac aat gat gat gca   915
Trp Ile Gly Ser Asp Cys Asn Thr Tyr Phe Pro His Asn Asp Asp Ala
            285                 290                 295

aag act ggt atc act ctg tct ggc aat ggt gtt gct ggc acc aat gga   963
Lys Thr Gly Ile Thr Leu Ser Gly Asn Gly Val Ala Gly Thr Asn Gly
        300                 305                 310

tct tgt gac aaa act cac aca tgc cca ccg tgc cca gca cct gaa gcc   1011
Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala
    315                 320                 325

gag ggc gcg ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc   1059
Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
330                 335                 340                 345

ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg   1107
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
                350                 355                 360

agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg   1155
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
            365                 370                 375

gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac agc   1203
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
        380                 385                 390

acg tac cgg gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg   1251
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
    395                 400                 405

aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc   1299
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
410                 415                 420                 425

ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca   1347
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
                430                 435                 440

cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag   1395
Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
            445                 450                 455

gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc   1443
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
        460                 465                 470

gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg   1491
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
    475                 480                 485

cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc   1539
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
490                 495                 500                 505

acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc   1587
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
                510                 515                 520

gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc   1635
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
            525                 530                 535

ctg tct ccg ggt aaa tga actagagcgg ccgctacaga t                  1674
```

Leu Ser Pro Gly Lys
540

<210> 16
<211> 542
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: fusion polypeptide

<400> 16
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15
Gly Ser Thr Gly Thr Ser Cys Gly Asn Gly Phe Ile Glu Thr Gly Glu
20 25 30
Glu Cys Asp Cys Gly Thr Pro Ala Glu Cys Val Leu Glu Gly Ala Glu
35 40 45
Cys Cys Lys Lys Cys Thr Leu Thr Gln Asp Ser Gln Cys Ser Asp Gly
50 55 60
Leu Cys Cys Lys Lys Cys Lys Phe Gln Pro Met Gly Thr Val Cys Arg
65 70 75 80
Glu Ala Val Asn Asp Cys Asp Ile Arg Glu Thr Cys Ser Gly Asn Ser
85 90 95
Ser Gln Cys Ala Pro Asn Ile His Lys Met Asp Gly Tyr Ser Cys Asp
100 105 110
Gly Val Gln Gly Ile Cys Phe Gly Gly Arg Cys Lys Thr Arg Asp Arg
115 120 125
Gln Cys Lys Tyr Ile Trp Gly Gln Lys Val Thr Ala Ser Asp Lys Tyr
130 135 140
Cys Tyr Glu Lys Leu Asn Ile Glu Gly Thr Glu Lys Gly Asn Cys Gly
145 150 155 160
Lys Asp Lys Asp Thr Trp Ile Gln Cys Asn Lys Arg Asp Val Leu Cys
165 170 175
Gly Tyr Leu Leu Cys Thr Asn Ile Gly Asn Ile Pro Arg Leu Gly Glu
180 185 190
Leu Asp Gly Glu Ile Thr Ser Thr Leu Val Val Gln Gln Gly Arg Thr
195 200 205
Leu Asn Cys Ser Gly Gly His Val Lys Leu Glu Glu Asp Val Asp Leu
210 215 220
Gly Tyr Val Glu Asp Gly Thr Pro Cys Gly Pro Gln Met Met Cys Leu
225 230 235 240
Glu His Arg Cys Leu Pro Val Ala Ser Phe Asn Phe Ser Thr Cys Leu
245 250 255
Ser Ser Lys Glu Gly Thr Ile Cys Ser Gly Asn Gly Val Cys Ser Asn
260 265 270
Glu Leu Lys Cys Val Cys Asn Arg His Trp Ile Gly Ser Asp Cys Asn
275 280 285
Thr Tyr Phe Pro His Asn Asp Asp Ala Lys Thr Gly Ile Thr Leu Ser
290 295 300
Gly Asn Gly Val Ala Gly Thr Asn Gly Ser Cys Asp Lys Thr His Thr
305 310 315 320
Cys Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe
325 330 335
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
340 345 350
Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
355 360 365
Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
370 375 380
Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
385 390 395 400
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
405 410 415
Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
420 425 430

```
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
        435                 440                 445
Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
    450                 455                 460
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
465                 470                 475                 480
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
                485                 490                 495
Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
            500                 505                 510
Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
        515                 520                 525
Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
    530                 535                 540
```

<210> 17
<211> 1668
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: fusion polypeptide

<220>
<221> CDS
<222> (25)..(1647)

<400> 17

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gtcgacccaa gctggctagc cacc atg gag aca gac aca ctc ctg cta tgg    51
                           Met Glu Thr Asp Thr Leu Leu Leu Trp
                             1               5

gta ctg ctg ctc tgg gtt cca ggt tcc act ggt act agt tgt gga aat   99
Val Leu Leu Leu Trp Val Pro Gly Ser Thr Gly Thr Ser Cys Gly Asn
 10                  15                  20                  25

gga tac gtc gaa gct ggg gag gag tgt gat tgt ggt ttt cat gtg gaa   147
Gly Tyr Val Glu Ala Gly Glu Glu Cys Asp Cys Gly Phe His Val Glu
                 30                  35                  40

tgc tat gga tta tgc tgt aag aaa tgt tcc ctc tcc aac ggg gct cac   195
Cys Tyr Gly Leu Cys Cys Lys Lys Cys Ser Leu Ser Asn Gly Ala His
             45                  50                  55

tgc agc gac ggg ccc tgc tgt aac aat acc tca tgt ctt ttt cag cca   243
Cys Ser Asp Gly Pro Cys Cys Asn Asn Thr Ser Cys Leu Phe Gln Pro
         60                  65                  70

cga ggg tat gaa tgc cgg gat gct gtg aac gag tgt gat att act gaa   291
Arg Gly Tyr Glu Cys Arg Asp Ala Val Asn Glu Cys Asp Ile Thr Glu
     75                  80                  85

tat tgt act gga gac tct ggt cag tgc cca cca aat ctt cat aag caa   339
Tyr Cys Thr Gly Asp Ser Gly Gln Cys Pro Pro Asn Leu His Lys Gln
 90                  95                  100                 105

gac gga tat gca tgc aat caa aat cag ggc cgc tgc tac aat ggc gag   387
Asp Gly Tyr Ala Cys Asn Gln Asn Gln Gly Arg Cys Tyr Asn Gly Glu
                110                 115                 120

tgc aag gcc aga gac aac cag tgt cag tac atc tgg gga aca aag gct   435
Cys Lys Ala Arg Asp Asn Gln Cys Gln Tyr Ile Trp Gly Thr Lys Ala
            125                 130                 135
```

```
gca ggg tct gac aag ttc tgc tat gaa aag ctg aat aca gaa ggc act   483
Ala Gly Ser Asp Lys Phe Cys Tyr Glu Lys Leu Asn Thr Glu Gly Thr
        140                 145                 150

gag aag gga aac tgc ggg aag gat gga gac cgg tgg att cag tgc agc   531
Glu Lys Gly Asn Cys Gly Lys Asp Gly Asp Arg Trp Ile Gln Cys Ser
    155                 160                 165

aaa cat gat gtg ttc tgt gga ttc tta ctc tgt acc aat ctt act cga   579
Lys His Asp Val Phe Cys Gly Phe Leu Leu Cys Thr Asn Leu Thr Arg
170                 175                 180                 185

gct cca cgt att ggt caa ctt cag ggt gag atc att cca act tcc ttc   627
Ala Pro Arg Ile Gly Gln Leu Gln Gly Glu Ile Ile Pro Thr Ser Phe
                190                 195                 200

tac cat caa ggc cgg gtg att gac tgc agt ggt gcc cat gta gtt tta   675
Tyr His Gln Gly Arg Val Ile Asp Cys Ser Gly Ala His Val Val Leu
            205                 210                 215

gat gat gat acg gat gtg ggc tat gta gaa gat gga acg cca tgt ggc   723
Asp Asp Asp Thr Asp Val Gly Tyr Val Glu Asp Gly Thr Pro Cys Gly
        220                 225                 230

ccg tct atg atg tgt tta gat cgg aag tgc cta caa att caa gcc cta   771
Pro Ser Met Met Cys Leu Asp Arg Lys Cys Leu Gln Ile Gln Ala Leu
    235                 240                 245

aat atg agc agc tgt cca ctc gat tcc aag ggt aaa gtc tgt tcg ggc   819
Asn Met Ser Ser Cys Pro Leu Asp Ser Lys Gly Lys Val Cys Ser Gly
250                 255                 260                 265

cat ggg gtg tgt agt aat gaa gcc acc tgc att tgt gat ttc acc tgg   867
His Gly Val Cys Ser Asn Glu Ala Thr Cys Ile Cys Asp Phe Thr Trp
                270                 275                 280

gca ggg aca gat tgc agt atc cgg gat cca gtt agg aac ctt cac ccc   915
Ala Gly Thr Asp Cys Ser Ile Arg Asp Pro Val Arg Asn Leu His Pro
            285                 290                 295

ccc aag gat gaa gga ccc aag ggt cct agt gcc acc aat aga tct tgt   963
Pro Lys Asp Glu Gly Pro Lys Gly Pro Ser Ala Thr Asn Arg Ser Cys
        300                 305                 310

gac aaa act cac aca tgc cca ccg tgc cca gca cct gaa gcc gag ggc   1011
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly
    315                 320                 325

gcg ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg   1059
Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
330                 335                 340                 345

atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac   1107
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
                350                 355                 360

gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg   1155
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
            365                 370                 375

cat aat gcc aag aca aag ccg cgg gag gag cag tac aac agc acg tac   1203
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
        380                 385                 390

cgg gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc   1251
```

```
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
    395                 400                 405

aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc   1299
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
410                 415                 420                 425

gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg   1347
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
                430                 435                 440

tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc   1395
Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
            445                 450                 455

ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag   1443
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
        460                 465                 470

tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc   1491
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
    475                 480                 485

gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg   1539
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
490                 495                 500                 505

gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg   1587
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
                510                 515                 520

cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct   1635
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
            525                 530                 535

ccg ggt aaa tga actagagcgg ccgctacaga t                           1668
Pro Gly Lys
        540
```

<210> 18
<211> 540
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: fusion polypeptide

<400> 18

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Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
  1               5                  10                  15
Gly Ser Thr Gly Thr Ser Cys Gly Asn Gly Tyr Val Glu Ala Gly Glu
             20                  25                  30
Glu Cys Asp Cys Gly Phe His Val Glu Cys Tyr Gly Leu Cys Cys Lys
         35                  40                  45
Lys Cys Ser Leu Ser Asn Gly Ala His Cys Ser Asp Gly Pro Cys Cys
     50                  55                  60
Asn Asn Thr Ser Cys Leu Phe Gln Pro Arg Gly Tyr Glu Cys Arg Asp
 65                  70                  75                  80
Ala Val Asn Glu Cys Asp Ile Thr Glu Tyr Cys Thr Gly Asp Ser Gly
                 85                  90                  95
Gln Cys Pro Pro Asn Leu His Lys Gln Asp Gly Tyr Ala Cys Asn Gln
            100                 105                 110
Asn Gln Gly Arg Cys Tyr Asn Gly Glu Cys Lys Ala Arg Asp Asn Gln
        115                 120                 125
Cys Gln Tyr Ile Trp Gly Thr Lys Ala Ala Gly Ser Asp Lys Phe Cys
    130                 135                 140
```

```
Tyr Glu Lys Leu Asn Thr Glu Gly Thr Glu Lys Gly Asn Cys Gly Lys
145                 150                 155                 160
Asp Gly Asp Arg Trp Ile Gln Cys Ser Lys His Asp Val Phe Cys Gly
                165                 170                 175
Phe Leu Leu Cys Thr Asn Leu Thr Arg Ala Pro Arg Ile Gly Gln Leu
            180                 185                 190
Gln Gly Glu Ile Ile Pro Thr Ser Phe Tyr His Gln Gly Arg Val Ile
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Asp Cys Ser Gly Ala His Val Val Leu Asp Asp Asp Thr Asp Val Gly
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Tyr Val Glu Asp Gly Thr Pro Cys Gly Pro Ser Met Met Cys Leu Asp
225                 230                 235                 240
Arg Lys Cys Leu Gln Ile Gln Ala Leu Asn Met Ser Ser Cys Pro Leu
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Asp Ser Lys Gly Lys Val Cys Ser Gly His Gly Val Cys Ser Asn Glu
            260                 265                 270
Ala Thr Cys Ile Cys Asp Phe Thr Trp Ala Gly Thr Asp Cys Ser Ile
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Arg Asp Pro Val Arg Asn Leu His Pro Pro Lys Asp Glu Gly Pro Lys
    290                 295                 300
Gly Pro Ser Ala Thr Asn Arg Ser Cys Asp Lys Thr His Thr Cys Pro
305                 310                 315                 320
Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe
                325                 330                 335
Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
            340                 345                 350
Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
        355                 360                 365
Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
    370                 375                 380
Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
385                 390                 395                 400
Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
                405                 410                 415
Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
            420                 425                 430
Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
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Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
    450                 455                 460
Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
465                 470                 475                 480
Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
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20 25 30

Xaa Xaa Xaa Xaa Xaa Cys Cys Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa
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atg gag aca gac aca ctc ctg cta tgg gta ctg ctg ctc tgg gtt cca   165
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
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ggt tcc act ggt act agt tgt ggg aat ggt gtg gtt gaa gaa gga gaa   213
Gly Ser Thr Gly Thr Ser Cys Gly Asn Gly Val Val Glu Glu Gly Glu
             20                  25                  30

gag tgt gac tgt gga cct tta aag cat tgt gca aaa gat ccc tgc tgt   261
Glu Cys Asp Cys Gly Pro Leu Lys His Cys Ala Lys Asp Pro Cys Cys
         35                  40                  45

ctg tca aat tgc act ctg act gat ggt tct act tgt gct ttt ggg ctt   309
Leu Ser Asn Cys Thr Leu Thr Asp Gly Ser Thr Cys Ala Phe Gly Leu
     50                  55                  60

tgt tgc aaa gac tgc aag ttc cta cca tca ggg aaa gtg tgt aga aag   357
Cys Cys Lys Asp Cys Lys Phe Leu Pro Ser Gly Lys Val Cys Arg Lys
 65                  70                  75                  80

gag gtc aat gaa tgt gat ctt cca gag tgg tgc aat ggt act tcc cat   405
Glu Val Asn Glu Cys Asp Leu Pro Glu Trp Cys Asn Gly Thr Ser His
                 85                  90                  95

aag tgc cca gat gac ttt tat gtg gaa gat gga att ccc tgt aag gag   453
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            100                 105                 110

agg ggc tac tgc tat gaa aag agc tgt cat gac cgc aat gaa cag tgt   501
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        115                 120                 125

agg agg att ttt ggt gca ggc gca aat act gca agt gag act tgc tac   549
Arg Arg Ile Phe Gly Ala Gly Ala Asn Thr Ala Ser Glu Thr Cys Tyr
    130                 135                 140

aaa gaa ttg aac acc tta ggt gac cgt gtt ggt cac tgt ggt atc aaa   597
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145                 150                 155                 160

aat gct aca tat ata aag tgt aat atc tca gat gtc cag tgt gga aga   645
Asn Ala Thr Tyr Ile Lys Cys Asn Ile Ser Asp Val Gln Cys Gly Arg
                165                 170                 175
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att cag tgt gag aat gtg aca gaa att ccc aat atg agt gat cat act   693
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act gtg cat tgg gct cgc ttc aat gac ata atg tgc tgg agt act gat   741
Thr Val His Trp Ala Arg Phe Asn Asp Ile Met Cys Trp Ser Thr Asp
        195                 200                 205

tac cat ttg ggg atg aag gga cct gat att ggt gaa gtg aaa gat gga   789
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    210                 215                 220

aca gag tgt ggg ata gat cat ata tgc atc cac agg cac tgt gtc cat   837
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225                 230                 235                 240

ata acc atc ttg aat agt aat tgc tca cct gca ttt tgt aac aag agg   885
Ile Thr Ile Leu Asn Ser Asn Cys Ser Pro Ala Phe Cys Asn Lys Arg
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ggc atc tgc aac aat aaa cat cac tgc cat tgc aat tat ctg tgg gac   933
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cct ccc aac tgc ctg ata aaa ggc tat gga ggt agt gtt gac agt ggc   981
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        275                 280                 285

cca ccc cct aag aga aag aag aaa aag aag aga tct tgt gac aaa act  1029
Pro Pro Pro Lys Arg Lys Lys Lys Lys Lys Arg Ser Cys Asp Lys Thr
    290                 295                 300

cac aca tgc cca ccg tgc cca gca cct gaa gcc gag ggc gcg ccg tca  1077
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305                 310                 315                 320

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acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct  1173
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
            340                 345                 350

gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc  1221
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
        355                 360                 365

aag aca aag ccg cgg gag gag cag tac aac agc acg tac cgg gtg gtc  1269
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
    370                 375                 380

agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac  1317
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385                 390                 395                 400

aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc  1365
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
                405                 410                 415

atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg  1413
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            420                 425                 430

ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc  1461
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    450                 455                 460

aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac   1557
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465                 470                 475                 480

tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag agc   1605
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Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
        500                 505                 510

ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa   1701
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      polypeptide

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Glu Cys Asp Cys Gly Pro Leu Lys His Cys Ala Lys Asp Pro Cys Cys
         35                  40                  45
Leu Ser Asn Cys Thr Leu Thr Asp Gly Ser Thr Cys Ala Phe Gly Leu
     50                  55                  60
Cys Cys Lys Asp Cys Lys Phe Leu Pro Ser Gly Lys Val Cys Arg Lys
 65                  70                  75                  80
Glu Val Asn Glu Cys Asp Leu Pro Glu Trp Cys Asn Gly Thr Ser His
                 85                  90                  95
Lys Cys Pro Asp Asp Phe Tyr Val Glu Asp Gly Ile Pro Cys Lys Glu
            100                 105                 110
Arg Gly Tyr Cys Tyr Glu Lys Ser Cys His Asp Arg Asn Glu Gln Cys
        115                 120                 125
Arg Arg Ile Phe Gly Ala Gly Ala Asn Thr Ala Ser Glu Thr Cys Tyr
    130                 135                 140
Lys Glu Leu Asn Thr Leu Gly Asp Arg Val Gly His Cys Gly Ile Lys
145                 150                 155                 160
Asn Ala Thr Tyr Ile Lys Cys Asn Ile Ser Asp Val Gln Cys Gly Arg
                165                 170                 175
Ile Gln Cys Glu Asn Val Thr Glu Ile Pro Asn Met Ser Asp His Thr
            180                 185                 190
Thr Val His Trp Ala Arg Phe Asn Asp Ile Met Cys Trp Ser Thr Asp
        195                 200                 205
Tyr His Leu Gly Met Lys Gly Pro Asp Ile Gly Glu Val Lys Asp Gly
    210                 215                 220
Thr Glu Cys Gly Ile Asp His Ile Cys Ile His Arg His Cys Val His
225                 230                 235                 240
Ile Thr Ile Leu Asn Ser Asn Cys Ser Pro Ala Phe Cys Asn Lys Arg
                245                 250                 255
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Gly Ile Cys Asn Asn Lys His His Cys His Cys Asn Tyr Leu Trp Asp
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Pro Pro Asn Cys Leu Ile Lys Gly Tyr Gly Gly Ser Val Asp Ser Gly
        275                 280                 285
Pro Pro Pro Lys Arg Lys Lys Lys Lys Lys Arg Ser Cys Asp Lys Thr
    290                 295                 300
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser
305                 310                 315                 320
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
                325                 330                 335
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
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Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
        355                 360                 365
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
    370                 375                 380
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
385                 390                 395                 400
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
                405                 410                 415
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
            420                 425                 430
Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys
        435                 440                 445
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
    450                 455                 460
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
465                 470                 475                 480
Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
                485                 490                 495
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
            500                 505                 510
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        515                 520                 525
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(71) Applicant *(for all designated States except US)*: IMMUNEX CORPORATION [US/US]; 51 University Street, Seattle, WA 98101 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: FANSLOW, William, C., III [US/US]; 404 S.W. 197th Street, Normandy Park, WA 98166 (US). CERRETTI, Douglas, Pat [US/US]; 1607 North 197th Place, Seattle, WA 98133 (US). POINDEXTER, Kurt, Matthew [US/US]; 9247 Interlake Avenue North, Apt. 2, Seattle, WA 98103 (US). BLACK, Roy, Alvin [US/US]; 8062 30th Avenue Northeast, Seattle, WA 98115 (US).

(74) Agent: SMITH, Julie, K.; 51 University Street, Seattle, WA 98101 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published:**
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

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*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

WO 01/62905 A3

(54) Title: INTEGRIN ANTAGONISTS

(57) Abstract: The present invention provides methods and compositions for inhibiting the biological activity of integrins, for inhibiting endothelial cell migration, and for inhibiting angiogenesis. In particular, the invention provides compositions comprising ADAM disintegrin domains and methods for using said compositions. In preferred embodiments the methods and compositions of the invention are used to inhibit angiogenesis and to treat diseases or conditions mediated by angiogenesis.

# INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 01/05701

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N9/64 C12N15/57 A61K38/16 A61P35/00 A61P37/00
A61P27/00 A61P17/02 C07K14/705

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EPO-Internal, WPI Data, PAJ, SCISEARCH, MEDLINE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | SCHLUESENER HERMANN J: "The disintegrin domain of ADAM 8 enhances protection against rat experimental autoimmune encephalomyelitis, neuritis and uveitis by a polyvalent autoantigen vaccine." JOURNAL OF NEUROIMMUNOLOGY, vol. 87, no. 1-2, 1 July 1998 (1998-07-01), pages 197-202, XP000926791 ISSN: 0165-5728 page 199 -page 201; figure 2A<br>---<br>-/-- | 1-3,16, 17,26 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 20 December 2001 | 16/01/2002 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | De Kok, A |

# INTERNATIONAL SEARCH REPORT

Inter ional Application No
PCT/US 01/05701

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | NATH DEEPA ET AL: "Interaction of metargidin (ADAM-15) with alphavbeta3 and alpha5beta1 integrins on different haemopoietic cells." JOURNAL OF CELL SCIENCE, vol. 112, no. 4, February 1999 (1999-02), pages 579-587, XP002186267 LONDON GB ISSN: 0021-9533 cited in the application the whole document, especially page 586, column 1 | 1-3, 7-18,27, 31,33-41 |
| Y | | 4 |
| A | | 35-42 |
| X | ZHANG XI-PING ET AL: "Specific interaction of the recombinant disintegrin-like domain of MDC-15 (metargidin, ADAM-15) with integrin alphavbeta3." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 13, 27 March 1998 (1998-03-27), pages 7345-7350, XP002186268 WASHINGTON US ISSN: 0021-9258 the whole document, especially page 7349, column 2, paragraph 2 | 1-3, 9-18,27, 31,33 |
| Y | SHEU J-R ET AL: "Inhibition of angiogenesis in vitro and in vivo: comparison of the relative activities of triflavin, an Arg-Gly-Asp-containing peptide and anti-alphavbeta3 integrin monoclonal antibody" BBA - GENERAL SUBJECTS, ELSEVIER SCIENCE PUBLISHERS, NL, vol. 1336, no. 3, 20 October 1997 (1997-10-20), pages 445-454, XP004276037 ISSN: 0304-4165 abstract | 4 |

-/--

# INTERNATIONAL SEARCH REPORT

Inter 'ional Application No
PCT/US 01/05701

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication,where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | TSELEPIS VICKY H ET AL: "An RGD to LDV motif conversion within the disintegrin kistrin generates an integrin antagonist that retains potency but exhibits altered receptor specificity: Evidence for a functional equivalence of acidic integrin-binding motifs" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 272, no. 34, 1997, pages 21341-21348, XP002149905 ISSN: 0021-9258 the whole document | 4 |
| A | WO 99 41388 A (IMMUNEX CORP) 19 August 1999 (1999-08-19) cited in the application the whole document | 1-42 |
| A | WO 99 23228 A (IMMUNEX CORP) 14 May 1999 (1999-05-14) cited in the application page 6, paragraph 2 page 8, paragraph 2 | 1-42 |
| A | WO 99 36549 A (IMMUNEX CORP ) 22 July 1999 (1999-07-22) cited in the application page 4, line 24 - line 30 page 7, line 25 -page 8, line 26 | 1-42 |
| P,X | WO 00 43493 A (HUMAN GENOME SCIENCES INC ) 27 July 2000 (2000-07-27) page 13, line 3 page 17, line 6 - line 7 page 196, line 31 -page 204, line 33 page 227 -page 234 examples 10,39,41-43,49 | 1-9, 11-29, 31,32, 34-42 |
| E | WO 01 74857 A (BRISTOL-MYERS SQUIBB CO) 11 October 2001 (2001-10-11) page 4, line 26 -page 6, line 16 page 7, line 11 -page 8, line 26 page 14, line 17 - line 34; example 12 | 1-18,20, 27,28, 30-42 |

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-3, 18-20, 26 completely and 5-17, 21-25 partly

   A method of antagonizing the binding of an integrin to its ligand, in vitro or in vivo, by administering an effective amount of an ADAM disintegrin domain polypeptide

2. Claims: 4, 28, 29 completely and 5-17, 21-25, 27 partly

   A method of inhibiting angiogenesis in a mammal comprising administering an ADAM disintegrin domain polypeptide which does not contain a RGD sequence

3. Claim : 27 partly and 30 completely

   A method for inhibiting the biological activity of alphaIIbetaI integrin comprising contacting the integrin with an ADAM-23 disintegrin polypeptide

4. Claim : 27 partly and 31 completely

   A method for inhibiting the biological activity of alphaVbetaI integrin comprising contacting the integrin with an ADAM disintegrin polypeptide and the ADAM is ADAM-15, -21, -22 or -23

5. Claim : 27 partly and 32 completely

   A method for inhibiting the biological activity of alphaVIbetaI or alphaVIbetaIV integrin comprising contacting the integrin with an ADAM disintegrin polypeptide and the ADAM is ADAM-10, -17, -22 or -23

6. Claim : 27 partly and 33 completely

   A method for inhibiting the biological activity of alphaVbetaV integrin comprising contacting the integrin with an ADAM disintegrin polypeptide and the ADAM is ADAM-10, -15 or -23

7. Claims: 34-42

   Methods for identifying compounds that modulate integrin biological activity

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-10 and 15-26 relate to a method defined by reference to the use of a compound having a desirable characteristic or property, namely having an "ADAM disintegrating domain".
The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the subject-matter of claims 11-14, insofar as those claims refer to amino acid or nucleotide sequences as identified in the sequence listing since fragments (claim 11b, 13b), variants (claim 11c) fusion proteins (claim 11d) or hybridizing nucleic acids (claim 14 c) retaining at least one 'ADAMdis' activity are not disclosed as well.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

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